EFFECT OF NITRIC OXIDE AND BRASSINOSTEROIDS ON THE SALINITY INDUCED CHANGES IN TOMATO (*LYCOPERSICON ESCUENTUM*)

ABSTRACT

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

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DEPARTMENT OF BOTANY
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ABSTRACT

Effect of nitric oxide and brassinosteroids on the salinity induced changes in tomato (*Lycopersicon Esculentum*)

SANGEETA YADAV

Abstract of the thesis, submitted to the Aligarh Muslim University, Aligarh, India, for the degree of Doctor of Philosophy in Botany, 2010.

Eight pot experiments were conducted during 2007-2009 to find out degree of tolerance to salinity in tomato (*Lycopersicon esculentum*) var. K-21 and to find out the most toxic concentration of salt as seed soaking (50, 100 or 150 mM) or amended in soil (2.9, 5.8 or 8.7 mg kg^{-1} of soil) of NaCl. Moreover, the response of tomato plant towards two brassinosteroid analogues, 28-homobrassinolide (HBL) and 24-epibrassinolide (EBL), and nitric oxide [source of nitric oxide is sodium nitroprusside (SNP)] were also studied. Brassinosteroid analogues were applied as foliar spray, whereas, nitric oxide through seed soaking. The salient features in each of the eight experiments are mentioned below.

Experiment 1

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in double distilled water (DDW), 50, 100 or 150 mM of NaCl for 8 hours. The treated seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. Twenty days after sowing (DAS), these treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS to assess growth, relative water content, photosynthetic attributes, SPAD chlorophyll, activity of nitrate reductase and carbonic anhydrase, proline content and antioxidative enzymes. Plants showed significantly different response to the different salt concentration. 150 mM NaCl concentration was found to be the most toxic. All the

above parameters except antioxidative enzymes and proline content, showed significant decrease in response to sodium chloride treatment. However, NaCl treatment resulted in a significant increase in the antioxidative enzymes and proline content and their values increased with the increasing concentration of salt.

Experiment 2

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var K-21 were sown in earthen pots containing 0, 2.9, 5.8 or 8.7 mg of NaCl/Kg of soil. These earthen pots were filled with study loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. The tomato plant showed significantly different response to different concentrations of salt. The highest level of salt (8.7 mg Kg^{-1}) was the most toxic. All the parameters except antioxidative enzymes and proline content showed a linear decrease as the level of the salt in the sol increased (2.9, 5.8 or 8.9 mg Kg^{-1} soil). The highest level of sodium chloride (8.7 mg Kg^{-1} soil) showed maximum antioxidative enzyme and that of proline content.

Experiment 3

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW, 10^{-4}, 10^{-5} or 10^{-6} M sodium nitroprusside (SNP) for 8 hours. The treated seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. After 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. Tomato plants showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 45 to 60 DAS. Treatment of SNP shows a different response and up to 10^{-5}M of SNP, most parameters increased. 10^{-6}M of SNP proved to be inhibitory.

Experiment 4

The surface sterilized seeds of tomato (*Lycopersicon eculentum*) var. K-21 were soaked in DDW for 8 hours. These seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. After 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The foliage of forty four days old seedlings were sprayed with DDW, 10^{-6}, 10^{-8} or 10^{-10} M of 28 homobrassinolide (HBL) or 24-epibrasinolide (EBL). The samples were collected at 45 and 60 DAS. The parameters studied were the same as in experiment 1. Tomato plants showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 45 to 60 DAS. The best response was obtained by 10^{-8}M of HBL/EBL. Out of the two brassinosteroid analogues (HBL/EBL), EBL was more effective than HBL.

Experiment 5

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW, 50, 100 or 150 mM NaCl for 8 hours. The treated seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The foliage of forty four day old plants was sprayed with DDW/aqueous solution of 10^{-8} M of HBL or EBL. The samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. Tomato plants showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 45 to 60 DAS. The follow up treatment of either of the brassinosteroid analogues (HBL/EBL) significantly neutralized the ill effect of salt. The level of proline and the activity of antioxidative enzymes increased in response to salt and hormone treatment. Out of the two brassinosteroid analogues (HBL/EBL), EBL was more effective than HBL.

Experiment 6

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were sown in earthen pots containing 0, 2.9, 5.8 or 8.7 mg NaCl kg^{-1} soil. These earthen pots were filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS the treated seedling were subsequently transplanted to the maintained pots. The foliage of forty four day old plants was sprayed with DDW/aqueous solution of 10^{-8}M of 28 homobrassinolide (HBL) and 24-epibrasinolide (EBL). The plant samples were collected at 45 and 60 DAS to study the parameters same as in experiment 1. All the parameters increased with the progress of the plant age. The foliar spray of HBL or EBL improved the values of all the parameters and neutralized the damaging effect of the salt. EBL was better promoter than HBL.

Experiment 7

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW, 50, 100 or 150 mM NaCl for 8 hours and then transferred to the solution of DDW or 10^{-5} M of SNP for 8 hours again. The seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. Plants showed significantly different response to the treatment. All the parameters increased as the growth progressed from 45 to 60 DAS. The ill effect generated by the lowest concentration of salt was completely neutralized by SNP.

Experiment 8

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW or SNP (10^{-5}M) for 8 hours. The treated seeds were sown in earthen pots containing 0, 2.9, 5.8 or 8.7 mg

NaCl Kg^{-1} soil. These earthen pots were filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS the treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS to study the characteristics studied in experiment 1. All the parameters increased with the progress of the plant age. The ill effect generated by the lowest concentration of salt was completely neutralized by SNP, whereas medium concentration of salt was partially neutralized.

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Dedicated

To

My Parents

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Certificate

This is to certify that the thesis entitled, **"Effect of nitric oxide and brassinosteroids on the salinity induced changes in tomato (*Lycopersicon esculentum*)"**, submitted for the degree of Doctor of Philosophy in Botany is a faithful record of the bonafide research work carried out at Aligarh Muslim University, Aligarh, India, by **Mrs. Sangeeta Yadav** under my guidance and supervision and that no part of it has been submitted for any other degree or diploma.

(Dr. Shamsul Hayat)
Research Supervisor

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Chapter – 1

INTRODUCTION

CHAPTER-1

INTRODUCTION

Lycopersicon esculentum (Tomato) is the member of the family Solanaceae. A small genus of annual or short lived perennial herbs, indigenous to the western regions of tropical South America. One species *Lycopersicon esculentum* is widely cultivated throughout the world. In India, tomatoes can be grown nearly throughout the year. Favourable climatic conditions are available in one or the other part of the country.

Tomato plants are perennial dicot, typically reaching to 1.3 metres (3-10 ft) in height, it has a weak woody stem that often winds over other plants. The leaves are 10-25 centimeters (4 to 10 inch) long, odd pinnates, with 5-9 leaflets on petioles, each leaflet is up to 8 centimetres (3 inch) long, with a serrated margin; both the stem and leaves possess granular hairs. The flowers are 1-2 centimetres (0.4-0.8 inch) across, yellow with five pointed lobes on the corolla, they are borned in a cyme of 3-12 flowers together. Tomato fruit is classified as a berry. As a true fruit, it develops from the inferior ovary of the plants after fertilization; its flesh comprises the pericarp walls. Fruits contain hollow spaces full of seeds and pulp, called locular cavities. Fruits show slight variations, among cultivated species, according to the type. The seeds need to be picked from a mature fruits, and be dried/fermented before germination.

The tomato is grown worldwide for its edible fruits, with thousands of cultivars having been selected with varying fruit types and adjustability in different climatic conditions. The tomato plant requires a warm climate with plenty of sunshine and adequate moisture. It does not tolerate frost. It can be cultivated under irrigation in

arid tropics, but hot and dry or hot and humid conditions do not favour its growth. High humidity with high temperature renders the plant susceptible to foliage diseases. For the proper development of colour in the fruit, warm sunny days and moderately cool nights are necessary. As in most sectors of agriculture, there is increasing demand for tomato in developed and/or developing counties. According to FAOSTAT, 125 million tons of tomato was produced in the world in 2005. China being the largest producer, accounted for about one quarter of the global output followed by United States (11.0 million tons), Turkey (2.7 million tons) and India (7.6 million tons).

The chemical composition of tomato varies with variety and the stage of harvest. The pulp constitutes 85.4% of the whole fruit and contains 6-7% total solids. The principal organic acid present in the tomato fruit is citric acid but maleic acid also occurs in appreciable amounts. Carotenoids, β-carotene and lycopene constitute the chief colouring matter of tomato fruits; their concentration in the fruit depends on the variety and the stage of ripeness.

Tomatoes are now eaten freely throughout the world, and their consumption is believed to benefit the heart among other things. They contain lycopene, one of the most powerful natural antioxidants, which especially in the cooked tomatoes helps to prevent prostate cancer. Lycopene has also been shown to improve skin's ability to protect against harmful UV rays. Moreover, tomato is also used as tomato sauce, tomato soup, tomato juice. Unripe green tomato can also be breaded and fried to make salsa or pickled.

Soil salinity has become a serious environmental problem which affects the growth and productivity of many crops (Koca *et al.*, 2007). About 20% of the world's cultivated land area and 50% of all irrigated lands are affected by salinity (Moud and

Maghsoudi, 2008). High salt content affects the physiology of plants, both at the cellular as well as whole plant levels. Ionic imbalance occurs in the cells due to excessive accumulation of Na^+ and Cl^- ions that reduce the uptake of mineral nutrients such as K^+, Ca^{2+} and Mn^{2+} (Bayuelo-Jimenez *et al.*, 2003). Excess amount of sodium ions in cells cause enzyme inhibition and metabolic dysfunction such as degradation of photosynthetic pigments (Chaves *et al.*, 2009). Photosynthesis is one of the most severely affected processes during salinity stress which is mediated through a decrease in stomatal conductance (Parida *et al.*, 2004), internal CO_2 partial pressure and stomatal opening that affect gaseous exchange (Iyenger and Reddy, 1996). The decrease in photosynthesis under saline conditions is considered as one of the most important factor responsible for reduced plant growth and productivity (Manikandan and Desingh, 2009).

According to the incapacity to grow on high salt medium, plants have been classified as glycophytes or halophytes. Most cultivated plants are glycophytes and cannot tolerate salt stress (Sairam and Tyagi, 2004). The deleterious effects of salinity on plant growth are associated with: (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt effect) or (4) a combination of these factors (Marschner, 1995). During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolism are affected. The earliest response is a reduction in the rate of leaf surface expansion followed by its cessation as stress intensifies, but growth resumes when the stress is relieved (Parida and Das, 2005). Soil salinity causes a lower rate of photosynthesis by decreasing the chlorophyll content, the activity of rubisco (Soussi *et al.*, 1998) and the closure of stomata thereby, decreases partial CO_2 pressure (Bethke and Drew, 1992). Salinity reduces

plant productivity first by reducing plant growth during the phase of osmotic stress and subsequently by inducing leaf senescence during the phase of toxicity when excessive salt is accumulated in transpiring leaves (Munns, 2002). All of these cause adverse pleotropic effects on plants.

Nitric oxide (NO), at an elevated level acts as a toxic air pollutant, which is produced in the atmosphere mainly by electrical discharge, automobile engines and power plants (Stohr and Ullrich, 2002). In the air it is converted to nitric acid, which is an important component of acid rain and in association with nitrogen dioxide (NO_2) participates in ozone layer depletion (Kramlich and Linak, 1994). However, at a very low concentration, this inorganic free molecule has been described as gaseous phytohormone (Leshem, 2000) which is endogenously formed in many biological systems. NO production in plant tissues was first observed by Klepper (1975). Later on four different enzymatic pathways involved in NO production have been proposed; (a) nitric oxide synthase, (b) plasma membrane bound nitrate reductase, (c) mitochondrial electron transport chain and (d) non-enzymatic reactions (Durzan and Pedroso, 2002; Guo *et al.*, 2003; Hayat *et al.*, 2010b). Nitric oxide is a signaling plant growth regulator (Beligni and Lamittina, 2000; Stohr and Stremlau, 2005) that acts mainly against oxidative stress (Neill *et al.*, 2003). At low concentrations of NO either endogenously produced or exogenously applied, it exerts a significant growth promoting effects (Leshem, 1996) and acts as an intra and intercellular messenger and a functional metabolite, involved in the regulation of diverse biochemical and physiological processes in plants (Hayat *et al.*, 2010b). The processes regulated by NO include seed germination, growth and development (Hayat *et al.*, 2009), apoptosis, hypersensitive response and phytoalexin production (Zhang *et al.*, 2005; Besson-Bard *et al.*, 2008). Root organogenesis, hypocotyl growth, defense responses

and stomatal closure are the other responses assigned to NO (Chaki *et al.*, 2009).

The biological activities of NO are diverse, concentration dependent (Hayat *et al.*, 2010b) and are exerted on phylogenetically distant species that opens a fantastic window for yet unexplored field of NO's function in plant kingdom.

In the recent past, Brassinosteroids (BRs) have emerged as a new paradigm in the category of phytohormones. Like other plant hormones (auxins, gibberellins, cytokinins and abscisic acid), BRs, were shown to participate at very low concentration in the control of numerous processes associated with plant growth and development (Mandava, 1988; Friedrichsen and Chory, 2001; Bajguz and Hayat, 2009). Brassinosteroids have the ability to cause cell elongation and cell division in stems, inhibit root growth, promote xylem differentiation, and abscission (Mandava, 1988; Nemhauser *et al.*, 2004). They have also been noted to control several other process in plants, such as induced synthesis of nucleic acid and protein synthesis (Khripach *et al.*, 2003), activation of several enzymes (Hasan *et al.*, 2008), photosynthesis (Hayat *et al.*, 2007a) and increased fruit set (Kamuro and Takatsuto, 1999; Ali *et al.*, 2006). Increased stress tolerance in the plants is another role assigned to brassinosteroids (Clouse and Sasse, 1998). Among abiotic stresses, BR has been reported to counter high and low temperature stress (Kulaeva *et al.*, 1991; Wilen *et al.*, 1995), moisture stress (Sairam, 1994; Hayat *et al.*, 2008), drought stress (Schilling *et al.*, 1991; Fariduddin *et al.*, 2009), heavy metal stress (Alam *et al.*, 2007; Hayat *et al.*, 2007a; Ali *et al.*, 2008a; Hasan *et al.*, 2008; Fariduddin *et al.*, 2009; Sharma *et al.*, 2007; Bhardwaj *et al.*, 2007), salinity stress (Ali *et al.*, 2007, 2008b) and nitrosative stress (Hayat *et al.*, 2010b).

Keeping in view the above roles assigned to BRs and nitric oxide and the ever increasing salinity stress in soil, the present research was designed with an objective

to relate changes in growth, photosynthetic parameters and the level of antioxidative enzymes, in salinized plants of *Lycopersicon esculentum* with the induced resistance and to neutralize the effects of salinity stress by the application of brassinosteroids and nitric oxide. The hypothesis that is put to trail is that the application of brassinosteroids and nitric oxide will ameliorate the toxic effects of salinity on the growth of the test plant, *Lycopersicon esculentum* which is widely consumed throughout the world and easily accepted by the local farmers as a cash crop.

The following objectives were kept in mind while planning the experiments.

1. To select the tolerant and resistant level of salinity on tomato among the two modes of application i.e. seed soaking and soil treatment.
2. To screen out the best concentration treatment of sodium nitroprusside by seed soaking for given tomato cultivars.
3. To find out the best concentration of HBL/EBL spray treatment for given tomato cultivar.
4. To assess the effect of foliage applied HBL/EBL (10^{-8} M) on tomato plants raised from the seeds, soaked in three concentration of NaCl solution.
5. To observe the effect of foliage applied HBL/EBL (10^{-8} M) on tomato plants fed with three levels of NaCl in the soil.
6. To assess the effect of nitric oxide treatment on tomato seeds soaked in three concentrations of NaCl solution.
7. To observe the effect of nitric oxide on tomato plants, raised in the soil treated with three doses of NaCl.
8. To select the metabolic and growth parameters showing maximum response to the treatment and that may be designated as a scale or forecasting further growth and crop productivity.

Chapter – 2

REVIEW OF LITERATURE

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CHAPTER – 2

REVIEW OF LITERATURE

2.1 SALINITY

Salinity is one of the most important abiotic stresses, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching (Zhao *et al.*, 2007). According to the Food and Agriculture Organization (FAO) Land and Nutrition Management Service (2008), over 6% of the world's land is affected by either salinity or sodicity which accounts for more than 800 million ha of land. Saline soils are defined by Ponnamperuma (1984) as those that contain sufficient salt in the root zone to impair the growth of crop plants. However, since salt injury depends on species, variety, growth stage, environmental factors, and nature of the salts, it is difficult to define saline soils precisely. The USDA Salinity Laboratory defines a saline soil as having an ECc of 4dSm^{-1} or more. ECc is the electrical conductivity of the 'saturated paste extract', that is, of the solution extracted from a soil sample after being mixed with sufficient water to produce a saturated paste. The most widely accepted definition of a saline soil has been adopted from FAO (1997) as one that has an electrical conductivity of the saturation extract (ECc) of 4 dSm^{-1} or more, and soils with ECc's exceeding 15 dSm^{-1} are considered strongly saline.

The common cations associated with salinity are Na^+, Ca^{2+} and Mg^{2+}, while the common anions are Cl^-, SO_4^{2-} and HCO_3^-. Since Na^+, in particular, causes deterioration of the physical structure of soil, and Na^+ and Cl^- both are toxic to plants, are therefore considered the most important ions (Dubey, 1997; Hasegawa *et al.*, 2000). The Na^+ toxicity of many crop plants is correlated with over accumulation of

Na^+ into shoot (Munns, 1993, 2002, Tester and Davenport, 2003; Moller and Tester, 2007). Historically, soils were classified as saline, sodic or saline-sodic based on the total concentration of salt and the ratio of Na^+ to Ca^{2+} and Mg^{2+} in the saturated extract of the soil (Dudley, 1994).

Salinity occurs through natural or human induced processes that result in the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth. Sodicity is a secondary result of salinity in clay soils, where leaching through either natural or human induced processes has washed soluble salts into the subsoil, and left sodium bound to the negative charges of the clay due to an increase in its concentration. There is competition for fresh water among the municipal, industrial and agricultural sectors in several regions. The consequence has been a decreased allocation of fresh water to agriculture (Tilman *et al.*, 2002).

2.1.1 Effect of salinity on plants

Salts in the soil water may inhibit plant growth for two reasons:

(i) The presence of salt in the soil solution reduces the ability of the plant to take up water, and this leads to reduction in growth rate. This is referred to as the osmotic or water deficit effect of salinity (physiological drought).

(ii) If excessive amount of salt enters the plant in its transpiration stream there will be injury to cells in the transpiring leaves and this may cause further reductions in growth. This is called the salt specific or ion-excess effect of salinity (Greenway and Munns, 1980).

According to Dubey (1997) and Yeo (1998) salt causes both ionic and osmotic effects on plants and most of the known responses of plants to salinity are linked to these effects. The general response of plants to salinity is reduction in growth

(Romero-Aranda *et al.*, 2001; Ghoulam *et al.*, 2002; Manikandan and Desingh, 2009). The initial and primary effect of salinity, especially at low to moderate concentrations, is due to its osmotic effects (Munns and Termant, 1986; Jacoby, 1994). Osmotic effects of salts on plants are a result of lowering of the soil water potential due to increasing solute concentration in the root zone (Plate I).

At high salinity, some symptoms of plant damage may be recognized, such as necrosis and leaf tip burn due to Na^+ or Cl^- ions (Wahome *et al.*, 2001). High ionic concentrations may disturb membrane integrity and function; interferes with internal solute balance and nutrient uptake, causing nutritional deficiency (Plate I) and symptoms similar to those that occur in the absence of salinity (Hasegawa *et al.*, 2000).

Sodium and chloride, usually the most prevalent ions in saline soils or water, account for most of the deleterious effects that can be related to specific ion toxicities (Levitt, 1980). The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties (Bolarin *et al.*, 1991; Ghoulam *et al.*, 2002). The severity of salinity response is also mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon *et al.*, 1994). Salt stress affects all the major processes such as growth, water relations, photosynthesis and mineral uptake.

2.1.2 Effect of salinity on plant growth

Salinity causes reduction in plant growth e.g. in tomato (Romero-Aranda *et al.*, 2001), cotton (Meloni *et al.*, 2001), sugarbeet (Ghoulam *et al.*, 2002), fingermillet (Manikendan and Desingh, 2009) and tomato (Li, 2002; Tantawy, 2007; Tantawy et al., 2009), potato (Daneshmand *et al.*, 2010). However, there are differences in tolerance to salinity among species and cultivars as well as among the different plant

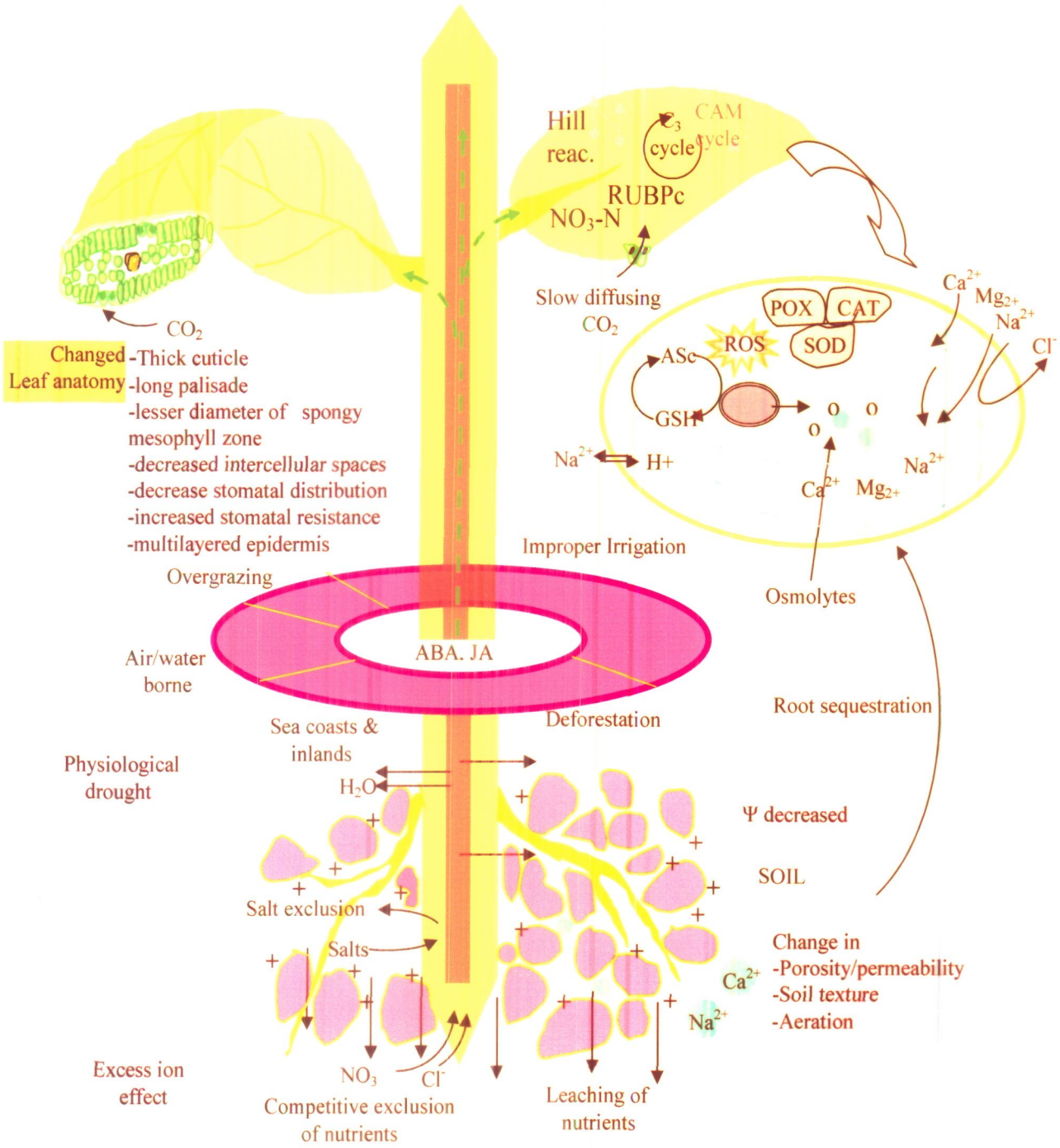


Plate-I. Causes of salinity and plant manifestations to salt stress

growth parameters, recorded. For instance, Aziz and Khan (2001) found that the optimum growth of *Rhizophora mucronata* plants was obtained at 50% seawater and declined with further increase in salinity while in *Alhagi pseudoalhagi* (a leguminous plant), total plant weight increased at Ca-salinity (50 mM NaCl) but decreased at high salinity (100 and 200 mM NaCl) (Kurban *et al.*, 1999). Application of NaCl (ECc 4.0 mS cm^{-1}) resulted in about 52, 50 and 55 % reduction in total nitrogen contents in mung-bean leaf, root and nodule, respectively (Chakrabarti and Mukherji, 2003). In sugar beet, leaf area, fresh and dry mass of leaves and roots were dramatically reduced at 200 mM NaCl, but leaf number was less affected (Ghoulam *et al.*, 2002). Manikandan and Desingh (2009) reported 40% and 52% reduction in two finger millet varieties (Intaf 25 and GPU-28) when subjected to 150 mM salinity. Fresh and dry weights of the whole plant were also markedly decreased under high salinity treatment in both the varieties of fingermillet. Fisarakis *et al.* (2001) working with Sultana vines recorded a larger decrease in accumulation of dry matter in shoots than in roots, particularly at high NaCl concentration, indicating partitioning of photo-assimilates in favour of roots. They proposed that the results may be due to a greater ability for osmotic adjustment under stress by roots.

2.1.3 Effect of salinity on water relations

According to Sohan *et al.* (1999) and Romero-Aranda *et al.* (2001) increase of salt in the root medium can lead to a decrease in leaf water potential and, hence, may affect many plant processes. Osmotic effects of salt on plants are as a result of lowering of the soil water potential due to increase in solute concentration in the root zone. At very low soil water potential, this condition interferes with plants ability to extract water from the soil and maintain turgor (Sohan *et al.*, 1999). However, at low or moderate salt concentration (higher soil water potential), plants adjust osmotically

(accumulate solutes) and maintain a potential gradient for the influx of water. Salt treatment causes a significant decrease in relative water content (RWC) in sugarbeet varieties (Ghoulam *et al.*, 2002) and *Brassica* species (Singh *et al.*, 2010). Water use efficiency (WUE) decreased with increasing levels of NaCl in *Thymus vulgaris* L. (Najafian *et al.*, 2009). According to Katerji *et al.* (1997), a decrease in RWC indicates a loss of turgor that results in limited water availability for cell extension processes.

2.1.4 Effect of salinity on leaf anatomy

Salinity has been reported to cause anatomical changes in the leaves of a number of plants. Salinity also reduces leaf area (Li and Stanghellini, 2001; Mulhulland *et al.*, 2002; Maggio *et al.*, 2004 and Agong *et al.*, 2004). Leaves of bean, cotton and *Atriplex* are reported to increase in epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter, and spongy cell diameter with increasing salinity (Longstreth and Nobel, 1979). In contrast, both epidermal and mesophyll thickness and intercellular spaces decreased significantly in NaCl treated leaves of the mangrove *Brugueira parviflora* (Parida *et al.*, 2004). In tomato plants salinity reduced the stomatal density (Parida *et al.*, 2004) (Plate I).

2.1.5 Effect of salinity on photosynthesis

Growth of the plants is dependent on photosynthesis and, therefore, environmental stresses affecting growth also affect photosynthesis (Salisbury and Ross, 1992; Dubey, 1997; Taiz and Zeiger, 1998; Manikandan and Desingh, 2009). Studies conducted by a number of authors with different plant species showed that photosynthetic capacity was suppressed by salinity (Dubey, 1997; Kao *et al.*, 2001; Ashraf, 2001; Romero-Aranda *et al.*, 2001; Chaves *et al.*, 2009). A positive association between photosynthetic rate and yield under saline conditions has been

found in different crops such as *Gossypium hirsutum* (Pettigrew and Meredith, 1994) and *Asparagus officinalis* (Faville *et al.*, 1999). Fisarakis *et al.* (2001) found that inhibition of vegetative growth in plants submitted to salinity was associated with a marked inhibition of photosynthesis. In contrast, there are many studies in which no or little association between growth and photosynthetic capacity is evident, as in *Triticum repens* (Rogers and Noble, 1992) and *Triticum aestivum* (Hawkins and Lewis, 1993).

The effect of salinity on photosynthetic rate depends on salt concentration and plant species. There is evidence that at low salt concentration salinity may stimulate photosynthesis. For instance, in *Brugueira parviflora*, Parida *et al.* (2004) reported that photosynthetic rate increased at low salinity and decreased at high salinity, whereas, stomatal conductance remained unchanged at low salinity and decreased at high salinity.

Iyengar and Reddy (1996) attributed the decrease in photosynthetic rate to salinity induced factors (Plate I):

(1) Dehydration of cell membranes which reduce their permeability to CO_2. High salt concentration in soil and water create high osmotic potential which reduces the availability of water to plants. Decrease in water potential causes osmotic stress, which reversibly inactivates photosynthetic electron transport via shrinkage of intercellular spaces.

(2) Salt toxicity caused particularly by Na^+ and Cl^- ions: According to Banuls *et al.* (1991), Cl^- inhibits photosynthetic rate through its inhibition of NO_3-N uptake by the roots. Fisarakis *et al.* (2001) found that NO_3-N was significantly reduced in salt-stressed Sultana vines and this reduction was correlated with photosynthetic

reduction. The reduced NO_3-N uptake combined with osmotic stress may explain the inhibitory effect of salinity on photosynthesis.

(3) Reduction of CO_2 supply because of the closure of stomata: The reduction in stomatal conductance results in restricting the availability of CO_2 for carboxylation reactions (Brugnoli and Bjorkman, 1992). Iyengar and Reddy (1996) reported that stomatal closure minimizes loss of water through transpiration and this affects light-harvesting and energy-conversion systems thus leading to alteration in chloroplast activity. Higher stomatal conductance in plants is known to increase CO_2 diffusion into the leaves and thereby favor higher photosynthetic rates. Higher net assimilation rates could in turn favor higher crop yields as was found by Radin *et al.* (1994) in Pima cotton (*Gossypium barbadense*). However, the results for photosynthetic rate and stomatal conductance presented by Ashraf (2001) for six Brassica species did not show any significant relationship. There are also reports of non stomatal inhibition of photosynthesis under salt stress. Iyengar and Reddy (1996) reported that this non stomatal inhibition is due to increased resistance to CO_2 diffusion in the liquid phase from the mesophyll wall to the site of CO_2 reduction in the chloroplast, and reduced efficiency of RuBPcase.

Other causes of reduced photosynthetic rates due to salinity have been identified by Iyengar and Reddy (1996) as: (4) enhanced senescence induced by salinity, (5) changes in enzyme activity induced by alterations in cytoplasmic structure and, (6) negative feedback by reduced sink activity. Although the rate of photosynthesis is reduced under salt stress, this is not the cause of reduction in the rate of cell expansion as suggested by several lines of evidence. According to Yeo *et al.* (1991) and Alarcon *et al.* (1994) growth is reduced more rapidly at lower concentrations of sodium in the leaf than photosynthesis. This means that plants can

withstand a certain loss in photosynthetic rate without any impact on growth. The relationship between photosynthesis and growth of plants under saline conditions is not well understood. Many changes take place in plants in order to enable them to tolerate saline conditions and maintain photosynthetic activity. An understanding of the mechanisms by which salinity affects photosynthesis would help in the improvement of growth conditions and crop yield and would provide useful tools for future genetic tailoring of plants.

2.1.6 Effect of salinity on ion levels and nutrient content

High salt (NaCl) uptake competes with the uptake of other nutrient ions, such as K^+, Ca^{2+}, N, P resulting in nutritional disorders and eventually, reduced yield and quality (Grattan and Grieve, 1999). Increased NaCl concentration has been reported to induce increase in Na^+ and Cl^- and decrease in Ca^{2+}, K^+ and Mg^{2+} level in a number of plants (Perez-Alfocea *et al.*, 1996; Khan *et al.*, 2000; Bayuelo-Jimenez *et al.*, 2003). Ghoulam *et al.* (2002) observed an increase in Na^+ and Cl^- content in the leaves and roots of sugarbeet with increasing NaCl concentration in the rooting medium. The K^+ content of the leaves decreased in response to NaCl, but that of roots was not affected by the salt treatment. A significant increase in Na^+ and Cl^- content in leaves, stem, and root of the mangrove (*Brugueira. parviflora*) has been reported without any significant alteration of the endogenous level of K^+ and Fe^{2+} in leaves (Parida *et al.*, 2004; Sridevi and Venkatesan, 2009). Decrease of Ca^{2+} and Mg^{2+} content of leaves have also been reported upon salt accumulation in this species.

Under salt stress conditions, the uptake of N by plants is generally affected. A number of studies have shown that salinity can reduce N accumulation in plants (Feigin *et al.*, 1991; Pardossi *et al.*, 1999; Silveira *et al.*, 2001). An increase in Cl^- uptake and accumulation has been observed to be accompanied by a decrease in shoot

NO_3^- concentration as in eggplant (Savvas and Lenz, 1996) and Sultana vines (Fisarakis *et al.*, 2001). Various authors have attributed this reduction to Cl^- antagonism of NO_3^- (Bar *et al.*, 1997) while others attributed the response to salinity effect on reduced water uptake (Lea-Cox and Syvertsen, 1993). The nitrate influx rate or the interaction between NO_3^- and Cl^- has been reported to be related to the salt tolerance of the species under investigation. Kafkafi *et al.* (1992) found that the more salt-tolerant tomato and melon cultivars had higher NO_3 flux rates than the more sensitive cultivars.

The effect of salinity on P concentration has been reported by Grattan and Grieve (1999) to be highly dependent on plant species, plant developmental stage, composition and level of salinity, and the concentration of P in the substrate. In most cases, salinity decreased the concentration of P in plant tissues (Sonneveld and de Kreij, 1999; Kaya *et al.*, 2001; Sridevi and Venkatesan, 2009), but the results of some studies indicate salinity either increased or had no effect on P uptake (Ansari, 1990). Salinity stress has stimulatory as well as inhibitory effects on the uptake of some micronutrients by plants. For a detailed review on this subject refer to Villora *et al.*, 1997; Grattan and Grieve, 1999. According to these authors nutrient imbalances may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant, or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant's internal requirement for that essential element.

2.1.7 Salinity tolerance

Salinity tolerance may be defined as the ability of a plant to grow and complete its life cycle under stressful salt conditions like NaCl or with association of other salts.

2.1.7.1 Morphological basis of salt tolerance

Two things are very important for the adaptation of a species under saline environment, one is control of water loss and the other is improved ionic balance. In many dicots and chenopod halophytes succulence is increased in response of salinity stress during adaptation. This succulence and enlargement of parenchyma cells are correlated as observed in *Atriplex* species (Greenway *et al.*, 1966). Plants under salt stress show succulence and xeromorphism e.g. NaCl presence caused succulence in cotton, tomato and *Salicornia* (Blits and Gallagher, 1991). It causes many structural changes as smaller leaves with reduction in number, fewer stomata, thickening of leaf cuticles and earlier lignifications of roots. These adaptations may play important role in maintaining tissue water content or succulence but depend upon the plant species and type, and extent of salinity stress (Poljakoff-Mayber, 1975).

The leaf water content in wheat are not affected by salinity but in case of radish and sunflower, salinity significantly decreases the leaf water content (Heikal, 1977). It has also been observed in many crop species that succulence is correlated with increase in total leaf volume (Jennings, 1976). This may happen by increasing the cell size, and in this way there is more accumulation of Na^+ and Cl^- in vacuole and finally vacuole-cytoplasm ratio is increased (Gorham *et al.*, 1985).

In some halophytes, special structures can be observed such as salt glands and bladders or trichomes. In these structures, excessive salt is accumulated which restricts the growing cells to the salt exposure (Flowers *et al.*, 1977; Greenway and Munns, 1980). Ions selection for NaCl via these special structures is highly selective (Luttge *et al.*, 1975). Salt glands have been found in wild rice; *Oryza coarctata* Roxb (Bal and Dutt, 1986).

2.1.7.2 Physiological basis of salt tolerance

Salts decrease water potential and create water deficit problem for plant growth. In such circumstances, plants must decrease inner water potential so that it may uptake water continuously. All plant species, whether halophytes or glycophytes, face two main problems when grown in saline soils, one is ion toxicity and the other is water deficit. The salt tolerance ability varies in different crop species. It is actually based on the type of species and the extent of stress. On the basis of tolerance level species have been divided into halophytes and glycophytes, former can tolerate high concentrations of salt while the latter are susceptible (Maas and Nieman, 1978). Halophytes have the ability to tolerate high concentrations of Na^+ and Cl^- by excluding toxic ions (Greenway and Munns, 1980; Jeschke, 1984; Lauchli, 1984). While in glycophytes, ions are present in the roots and do not move but halophytes move these ions towards shoot and this is the way, they tolerate the toxicity of ions (Flowers *et al.*, 1977).

Most of the halophytes respond to salinity through ion exclusion. In case of excessive NaCl, K^+ and Ca^+ ions are decreased (Lauchli, 1990; Cramer *et al.*, 1991). There are many mechanisms by which plants limit the Na^+ and Cl^- to reach the shoot. High K^+/Na^+ ratio in shoot is one of the mechanisms plants use to survive (Gorham *et al.*, 1985; Greenway and Munns, 1980; Aslam *et al.*, 1993; Gorham, 1994; Moller *et al.*, 2009). Pearson (1976) concluded that most of the Na^+ absorbed is retained in roots and lower part of the stem. Greenway (1973) called the exclusion an avoidance mechanism where roots remained impermeable to salts to some extent but, after attaining the threshold level, roots loose this ability and the existing salts burst and damage the shoot which leads to the death of plant. Under salt stress conditions, accumulation of salt in the plant is a must. Therefore, plants adapt different

mechanisms to get rid of it may be through glands (Flowers *et al.*, 1977) or via pumps at the plasma membrane of root cells (Jeschke, 1984). There are some plants which cope with the deleterious effects of salts by having more water to dilute the cell sap (Yeo and Flowers, 1984), while other plants distribute higher quantity of the salts in older leaves than in younger leaves (Yeo and Flowers, 1982). The intake of ions in older leaves is based on xylem transport while the export is through phloem. In case of younger leaves intake is through both xylem and phloem that is why, in younger leaves the load of ions is lesser as compared to older leaves (Flowers *et al.*, 1977; Greenway and Munns, 1980). Fenugreek plants develop a sodium inclusion mechanism by lowering the toxic concentration of ions in the cytoplasm by sequestration of Na^+ into the vacuole, which allows osmotic adjustment (Imed *et al.*, 2009). Salinity tolerance can be improved through conventional breeding and genetic engineering (Ashraf and Akram, 2009).

2.1.8 Induction of antioxidative enzymes by salinity

All environmental or manmade stresses have been reported to lead to the production of reactive oxygen species (ROS) that cause oxidative damage (Smirnoff, 1993; Schwanz *et al.*, 1996). Plants possess efficient systems for scavenging active oxygen species that protect them from destructive oxidative reactions (Foyer *et al.*, 1994). As part of this system, antioxidative enzymes are key elements in the defense mechanisms. Garratt *et al.* (2002) has listed some of these enzymes as catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD) and glutathione-S-transferase (GST). Superoxide dismutase that metabolizes oxygen radicals ($O_2^{\cdot}$) to hydrogen peroxide (H_2O_2), thus protecting cells from damage. Catalase, ascorbate peroxidase, and a variety of peroxidases catalyze the subsequent breakdown of H_2O_2 to water and oxygen (Chang *et al.*, 1984; Garratt *et al.*, 2002). Plants with high levels

of antioxidants have been reported to have greater resistance to this oxidative damage (Spychalla and Desborough, 1990).Garratt *et al.* (2002), Mittova *et al.* (2002, 2003) and Gobinathan *et* al. (2009) reported an increase in the activity of antioxidative enzymes in plants under salt stress. They found a correlation between these enzyme levels and salt tolerance. Similarly, many changes have been detected in the activity of antioxidant enzymes in plants exposed to salinity (Li *et al.*, 2010). The activity of antioxidant enzymes was reported to increase under saline conditions in shoot cultures of rice (Fadzilla *et al.*, 1997), wheat (Meneguzzo and Navarilzoo, 1999), pea (Hernandez *et al.*, 1999) and *Pennisetum typhoidies* (Gobinathan *et al.*, 2009), but decreased in wheat roots (Meneguzzo and Navarilzzo, 1999) or SOD was unaffected in cucumber (Lechno *et al.*, 1997). The variations in these observations may be due to the fact that the effects of salinity depend on a number of factors, for example, salt type, their concentration, plant genotype, growth stage and/or environmental conditions (Shannon *et al.*, 1994). The mechanism by which salinity affects the antioxidant responses is not yet clear. Meneguzzo and Navarilzzo (1999), however, proposed that it might be via the change in membrane integrity caused by high Na^+ to Ca^{2+} ratio.

2.1.9 Induction of plant hormones by salinity

The level of plant hormones such as ABA and cytokinins increases with high salt concentration (Vaidyanathan *et al.*, 1999). Abscisic acid (ABA) causes alteration in the expression of stress-induced genes which are predicted to play an important role in the mechanism of salt tolerance in rice (Gupta *et al.*, 1998). The inhibitory effects of NaCl on photosynthesis, growth and translocation of assimilates has been found to be alleviated by ABA (Popova *et al.*, 1995). Although the nature of ABA receptor(s) remains unknown, Leung and Giraudat (1998) pointed out that there is

substantial evidence of the involvement of ABA in reversible protein phosphorylation and modification of cytosolic calcium levels and pH. Chen *et al.* (2001) reported that the increase of Ca^{2+} uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer terms. ABA has been reported to reduce ethylene release and leaf abscission under salt stress in citrus probably by decreasing the accumulation of toxic Cl^- ions in leaves (Gomezcadenas *et al.*, 2002). Zhang *et al.*, (2009) proposed that the signaling cascades of ABA and BR primarily cross-talk after BR perception, but before their transcriptional activation. They explained a large proportion of BR-responsive genes are also regulated by ABA.

Exogenous treatment of 24-epibrassinosteroids (Ali *et al.*, 2008b) and salicylic acid (Yusuf *et al.*, 2008) protects *Brassica juncea* against salinity stress. SA has shown to enhance antioxidant enzymes activity (Yusuf *et al.*, 2008) and induce H_2O_2 production (Rao et al., 1997) to work as a signaling molecule. Pretreatment of H_2O_2 has been reported to induce salt tolerance in barley seedlings (Fedina *et al.*, 2009). Application of SA increased photosynthetic rates, mesophyll efficiency and WUE in salt stressed plants (Najafian *et* al., 2009). However, exogenous treatment of salicylic acid, H_2O_2 and Ca^{2+} induced salinity tolerance has been indicated its association with endogenous level of H_2O_2 homeostasis in naked oat seedlings (Xu et al., 2008). Higher levels of jasmonates were also found to accumulate in salt-tolerant tomato cultivars compared to the salt-sensitive ones (Hilda *et al.*, 2003). Jasmonates have been reported to have important roles in salt tolerance. However, it is yet not known whether SA and JA are synthesized *de-novo* in the osmotically stressed mesophyll cells of leaves under regulation of ABA or it is transported as methylated inactive

form from root to shoot. They are generally considered to mediate signaling, such as defense responses, flowering and senescence (Hilda *et al.*, 2003). However, factors involved in the salicylate and jasmonate signal-transduction pathway remain unclear (Plate I).

2.2 NITRIC OXIDE

Since, the last decade nitric oxide (NO) has been established as a novel biological messenger in plants and animals and received special attention from almost all the branches of science including medicine, bio-chemistry, physiology and genetics. The interest of biologist gained momentum when this highly reactive radical (NO) was identified as a potent endogenous vasodilators of the endothelium (Schmidt and Walter, 1994). The wide spread biological significance of NO was first recognized by Koshland (1992) who named NO, the free radical, as "Molecule of Year" and in 1998 the Nobal Prize in physiology and medicine was awarded for the discovery of NO as a biological mediator produced by mammalian cell.

The role of NO is not confined only to the animal kingdom but plants also have the ability to accumulate and metabolize atmospheric NO. Klepper (1975) for the first time observed the production of NO in soybean plant, treated with photosynthetic inhibitor herbicides (Klepper, 1978, 1979) or other chemicals (Klepper, 1991) as well as under anaerobic conditions (Klepper, 1987). In plants NO can be generated via enzymatic and non-enzymatic pathways. The enzymatic pathway is catalysed by cytosolic nitrate reductase (cNR), NO synthase (NOS) or NOS-like enzymes and nitrite: NO reductase (Ni-NOR) respectively. Non-enzymatic pathway is the nitrite dismutation to NO and nitrate at acidic pH value (Neill *et al.*, 2003; Graziano and Lamattina, 2005).

After the discovery of NO in plant, question arises, whether NO could be placed in the category of phytohormone or not because the classical concept of hormone includes three premises (Davies, 1995) (i) localized site of biosynthesis (ii) transport to target cells specially separated from the place of synthesis (iii) control of responses through changes in endogenous levels. NO had been found to formed mainly in actively growing tissues such as embryonic axes and cotyledons, and the level decreased in mature and senescent organs (Leshem *et al.*, 1998; Caro and Puntarulo, 1999). Secondly, the small size and high diffusion rate of NO through biological membranes mean that NO fits the premise that hormones are easily transported. In regard to the third statement, it is the sensitivity of the target cells, rather than the concentration of the plant hormone that defines the magnitude of a response (Trewaras and Malho, 1997), because of this concept some scientist decided to substitute the term hormone with the wider term 'plant growth regulator'. Later Belligni and Lamattina (2001) has described NO as a non-traditional regulator of plant growth.

Further investigation lead to the finding that NO is soluble in water and lipid. It can exist as three interchangeable forms: the radical ($NO^{\cdot}$), nitrosonium cation (NO^{+}); and nitroxyl anion (NO^{-}). Due to its highly lipophilic nature, NO may diffuse through membranes (Leshem, 1996) and act as inter- and intracellular messenger in many physiological functions. It plays a role in plant growth and development, seed germination, flowering, ripening, senescence of organs (Arasimowicz and Wiczorek, 2007) etc. Moreover like other phytohormones, NO also act in a concentration dependent manner.

Research on NO, in plants gained considerable attention in recent years and there is increasing evidence of a role of this molecule in plant. Therefore, in this

review an effort was made to cover the recent advances in chemical properties, mechanism of its bio-synthesis with special emphasis on the role of NO on the physiological and biochemical changes that occur in the plant under normal conditions due to the exogenously applied or endogenously produced NO, along with the cross-talk between NO and other phytohormones.

2.2.1 Chemistry

NO is a gaseous free radicals, its chemistry implicates interplay between the three redox-related species: nitric oxide radicals ($NO^{\cdot}$), nitrosonium cation (NO^{+}) and nitroxyl anion (NO^{-}). In biological system $NO^{\cdot}$ react rapidly with atmospheric oxygen (O_2), superoxide anion ($O_2^{\cdot-}$) and transition metals. The reaction of $NO^{\cdot}$ with $O_2^{\cdot-}$ results in the generation of NO_x compounds (including $NO_2^{\cdot}$, N_2O_3 and N_2O_4) which can either react with cellular amines and thiols, or simply hydrolyze to form the end metabolites, nitrite (NO_2^{-}) and nitrate (NO_3^{-}) (Wendehenne *et al.*,2001). The reaction of $NO^{\cdot}$ with O_2 yields peroxynitrite ($ONOO^{-}$), a powerful oxidant that mediate cellular injury. At physiological pH, $ONOO^{-}$ equilibrates rapidly with pernitrous acid (ONOOH) which, depending on its conformation, rapidly decomposes to NO_3^{-} or to the highly reactive hydroxyl radical $HO^{\cdot}$. $NO^{\cdot}$ also form complexes with the transition metals found in heme-or cluster-containing proteins, thus forming iron-nitrosyl complexes. This process alters the structure and function of the target proteins, as exemplified by the activation of soluble guanylate cyclase and the inhibition of aconitases.

In addition, $NO^{\cdot}$ is extremely susceptible to both oxidation and reduction. One electron oxidation of $NO^{\cdot}$ leads to NO^{+} (nitrosonium cation), while the product of one-electron reduction of $NO^{\cdot}$ is a nitroxyl radical (NO^{-}) (Stamler *et al.*,1992; Wojtaszek, 2000; Garcia-Mata and Lamattina, 2003). This oxidation can be supported

by Fe(III) containing metalloproteins (Stamler *et al.*,1992; Wojtaszek, 2000; Jasid *et al.*, 2008; Igamberdiev and Hill 2009). $NO^{\cdot}$ mediates electrophilic attack on reactive sulfur, oxygen, nitrogen and aromatic carbon centers, with thiols being the most reactive groups. This chemical process is referred to as nitrosation. Nitrosation of sulphydryl (S-nitrosation) centers of many enzymes or proteins have been described and the resulting chemical modification affects activity in many cases. Such modifications are reversible and protein S-nitrosation/denitrosation could represent an important mechanism for regulating signal transduction. The physiological significance of NO has been clarified. Some workers (Stamler *et al.*, 1992; Stamler,1994) suggest that it could act as a stabilized form of NO. NO is also believed to react with Fe (III) heme and to mediate sulphydryl oxidation of target proteins (Plate II).

2.2.2 Biosynthesis of nitric oxide

In biological systems, NO can be formed enzymatically and non-enzymatically. The enzyme responsible for NO generation in animal organisms is nitric oxide synthase (NOS). Although NOS-like activity has been detected widely in plants, but animal type NOS is still elusive. Recently, in pea seedlings, using the chemiluminescence assay Corpas *et al.* (2006) showed arginine-dependent NOS activity, which was constitutive, sensitive to an irreversible inhibitor of animal NOS and dependent on the plant organ and developmental stage. There have been a number of reports on the presence of NOS like activity in bacteria (Sudhamsu and Crane, 2009), unicellular eukaryotes (Ninnemann and Maier, 1996; Messner *et al.*, 2009) and plants (Besson-Bard *et al.*, 2008). Tossi *et al.* (2009) show apocyanin induce the accumulation of NO in leaves of maize seedling through a NOS-like activity.

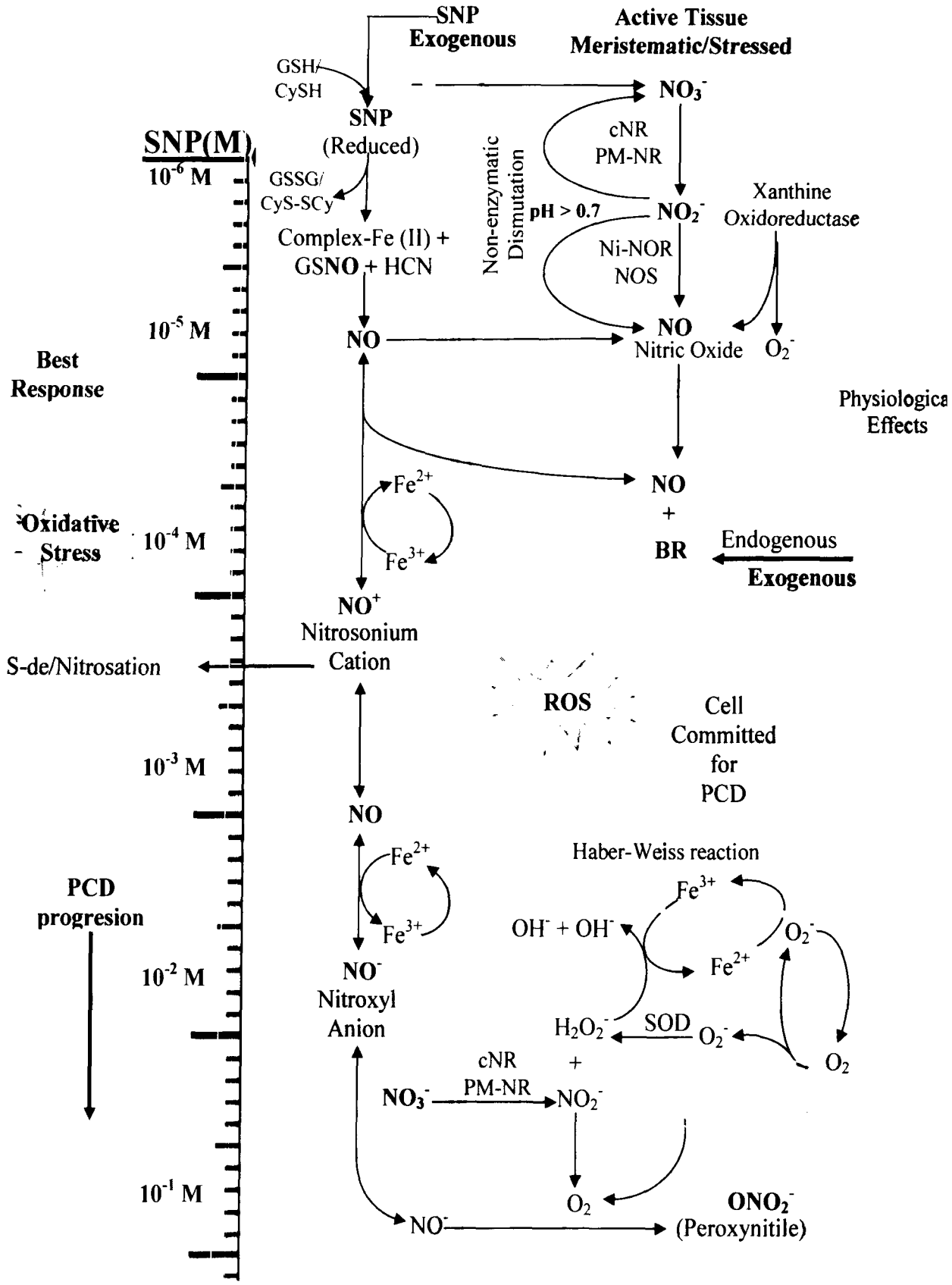


Plate-II. Effect of SNP/NO is dose dependent. NO reacts BR to ameliorate its excessive dose aftereffects. NO when interacts with ROS under suboptimal BR concentration, leads to PCD.

Gene encoding NOS-like proteins At NOS1 was isolated from the *Arabidopsis* genome, it was involved in the process of growth and hormonal signaling (Guo *et al.*, 2003). It was also found that At NOS1 may function as an NO source in the process of flowering control (He *et al.*, 2004) and in defense response induced by a lipopolysaccharide (Zeidler *et al.*, 2004). DNA sequencing analyses did not show affinity of the AtNOS1 protein to any of animal-origin NOS isoforms. However, the most recent studies have raised critical questions regarding the nature of At NOS1 (Zemojtel *et al.*, 2006; Crawford *et al.*, 2006). At NOS1 (Q664P9) and the orthologous genes from rice (Q6YPG5) and maize (AY110367) have been cloned, then after purification of recombinant protein, no NOS activity had be detected (Zemojtel *et al.*, 2006). Moreover, At NOS1 was identified as a member of GTP-binding family. Based on a report by Morimoto *et al.* (2002) in which bacterial protein Yqett, an ortholog of At NOS1 is defined as GTPase. It has been suggested that ATNOS1 might serve as GTPase involved in mitochondrial ribosome biogenesis and/or processes of translation (Zemojtel *et al.*, 2006) and in this case it might indirectly affect on NO synthesis. Later, it was proposed that the AtNOS1 gene be renamed ATNOA1- nitric oxide associated1 (Crawford *et al.*, 2006). Although the nature of AtNOA1 remains elusive and controversial (Zemojtel *et al.*, 2006; Crawford *et al.*, 2006; Guo *et al.*, 2006), there is no doubt that the identification of AtNOA1 protein and the Atnoa1 mutant has provided an effective way to genetically control *in-vivo* NOS activity and the endogenous NO levels as the Atnoa1 mutant have been consistently shown to have impaired *in-vivo* NOS activity and reduced endogenous NO levels (He *et al.*, 2004; Zeidler *et al.*, 2004; Guo *et al.*, 2006).

NO can also be produced by other enzymes apart from NOS, NR is one of them. NO generation via NR was demonstrated *in vitro* (Yamasaki and Sakihama,

2000). NR synthesizes this molecule from NO_2^-, at the participation of NAD(P)H (Kaiser and Huber., 2001) (Plate II). Transformation of NO_2^- to NO occurs most probably on a molybdenum cofactor. This synthesis was strictly dependent on nitrite and nitrate content in the tissue (Yamasaki and Sakihama, 2000; Kaiser and Huber, 2001; Rockel *et al.*, 2002). At a high *in vitro* nitrite concentration (e.g. 100 μM), NO synthesis constituted approximately 1% total NR reduction activity, whereas, *in-vivo* NO generation was estimated at 0.01-0.1% NR activity (Rockel *et al.*, 2002; Neill *et al.*, 2003). NO immediately reacts with O_2^-, forming peroxynitrite which contributes to a decrease of assayed NO concentration. Taking into consideration NO loss by the value of NO reaction with O_2^-, it was shown that the production of this signaling molecule in leaves of Vetch Chinese rose and *Arabidopsis thaliana* is almost 20 times higher than that assayed previously (Vanin *et al.*, 2004). NO production, dependent on NR activity, was also recorded in many other plant species, e.g. in cucumber (Haba *et al.*, 2001), sunflower, spinach, maize (Rockel *et al.*, 2002), *Arabidopsis* (Desikan *et al.*, 2002), tobacco (Planchet *et al.*, 2005), soyabean (Jasid *et al.*, 2006) as well as in *Chlamydomonas reinhardtii* (Sakihama *et al.*, 2002).

Xanthine oxidoreductase (XOR) is another Mo-Co containing enzyme which has been found to produce NO in plants as well as in animals. XOR occurs into two interconvertible forms: the superoxide producing xanthine oxidase and xanthine dehydrogenase (Palme *et al.*, 2002). XOR has been found to be present in pea leaf peroxisomes where the preponderant form of the enzyme is xanthine oxidase and only a 30% is present as xanthine dehydrogenase (Corpas *et al.*, 1997; Sandalio *et al.*, 1988). XOR can produce the free radicals O_2^- and NO during its catalytic reaction, depending on whether the oxygen tensions are high or low (Millar *et al.*, 1998; Godber *et al.*, 2000; Harrison, 2002). This property of producing O_2^- and NO radicals

confers XOR a key role as a source of signal molecule in plant cells (Corpas *et al.*, 2001). However, Planchet and Kaiser (2006b) were unable to observe any NO production from recombinant xanthine oxidase.

Another enzyme that can generate NO from nitrite, is a plasma membrane-bound enzyme of tobacco roots (Ni-NOR) (Stohr *et al.*, 2001). This enzyme has a higher molecular weight than nitrate reductase and still has to be characterized. Other good candidates for enzymatic generation of NO include: horseradish peroxidase (Huang *et al.*, 2002), cytochrome P450 (Boucher *et al.*, 1992a) catalase and haemoglobin (Boucher *et al.*, 1992b). The production of NO and citrulline by horseradish peroxidase from N-hydroxy-arginine (NOHA) and H_2O_2 was reported a decade ago (Boucher *et al.*, 1992a). More recently, horseradish peroxidase was also demonstrated to generate NO from hydroxyurea and H_2O_2 (Huang *et al.*, 2002).

Heme proteins that have been proposed as good candidates for the enzymatic generation of NO are cytochromes P450. These protein present in plants, and in animal systems have been shown to catalyze the oxidation of NOHA by NADPH and O_2 with generation of NO (Boucher *et al.*, 1992b; Mansuy and Boucher, 2002; Igamberdiev, 2009). Hemoglobin and catalase were also reported to produce NO and other nitrogen oxides by catalyzing the oxidation of NOHA by cumyl hydroperoxide (Boucher *et al.*, 1992a).

In plants, NO can also be generated by non-enzymatic mechanisms. Nitrification/denitrification cycle provide NO as a by-product of N_2O oxidation into the atmosphere (Wojtaszek, 2000). It is known that the non-enzymatic reduction of nitrite can lead to the formation of NO, and this reaction is favoured at acidic pH when nitrite can dismutate the NO and nitrate (Stohr and Ullrich, 2002). Nitrite can also be chemically reduced by ascorbic acid at pH 3-6 to yield NO and

dehydroascorbic acid (Henry *et al.*, 1997). This reaction could occur at microlocalized pH conditions in the chloroplast and apoplastic space where ascorbic acid is known to be present (Horemans *et al.*, 2000). In barley aleurone cells, NO can also be synthesized by reduction of nitrite by ascorbate at acidic pH (Beligni and Lamattina, 2002). Another non-enzymatic mechanism proposed of NO formation is the light mediated reduction of NO_2 by carotenoids (Cooney *et al.*, 1994).

2.2.3 Physiological role of NO

NO has emerged as an important signaling molecule associated with many biochemical and physiological processes in plants (Pagnussat *et al.*, 2002; Lamattina *et al.*, 2003; Stohr and Stremlau, 2005). NO was classified as a phytohormone which might function as a gaseous endogenous plant growth regulator (Leshem, 2000) and also as a non-traditional regulator of plant growth (Beligni and Lamattina, 2001). It has a huge capability to regulate diverse physiological processes, in a concentration dependent manner (Anderson and Mansfield, 1979; Gouvea *et al.*, 1997) such as root organogenesis, hypocotyls growth, defense responses, stomatal movement, apoptosis, hypersensitive responses growth and development, and phytoalaxin production etc. (Noritake *et al.*, 1996; Delledonne *et al.*, 1998; Durner *et al.*, 1998; Kim *et al.*, 1998; Durner and Klessig, 1999; Magalhaes *et al.*, 2000; Belgini and Lamatinna, 2000; Wendehenne *et al.*, 2001; Pagnussat *et al.*, 2002; Neill *et al.*, 2003; Chaki *et al.*, 2009) under different environmental conditions. Therefore, in the recent years, the role of NO in regulating various physiological and biochemical activities in plant has become an important area of research. In this section we discussed the role of NO on different processes of plant under normal condition (un-stressed plant) because so many articles regarding the role of NO in plant under differential abiotic and biotic stresses are already available.

2.2.3.1 Effect of NO on seed dormancy

Dormancy prevents seed germination under conditions that would otherwise allow germination. Many endogenous compounds reduce/break seed dormancy, among them one is nitrogen containing compounds that include nitrate, nitrite, hydroxylamine, azide and sodium nitroprusside (SNP). The ability of SNP to reduce seed dormancy in lettuce (Beligni and Lamattina, 2000), *Arabidopsis* (Batak *et al.*, 2002; Bethke *et al.*, 2004, 2006) and barley (Bethke *et al.*, 2004) led to the conclusion that NO played a role in dormancy breaking or germinating in these seeds. Moreover, stimulating effect of NO on seed germination had also been reported in other crops. NO stimulated seed germination in *Paulownia tomentosa* (Giba *et al.*, 1998) under normal conditions as well as in *Suaeda salsa* (WeiQiang *et al.*, 2005; Song *et al.*, 2009) and *Kosdeletzkya virginica* (Guo *et* al., 2009) under NaCl stress. The SNP (upto 0.8 mM) application promoted seed germination in lupin that was more pronounced after 18 and 24 hours and ceased after 48 hours (Kopyra and Gwozdz, 2003). Similarly in canola, NO stimulate seed germination in dose dependent manner; lower concentration of SNP (0.05 to 0.5 mM SNP) enhanced seed germination upto 18 h whereas high concentration (1 and 2 mM) inhibited it (Zanardo *et al.*, 2005). Furthermore, exogenous application of nitric oxide also promoted seed germination in maize (Zhang *et al.*, 2004) and pearl millet (Manjunathan *et al.*, 2008).

2.2.3.2 Effect of NO on growth

In rapidly growing pea seedlings a treatment of NO showed a dual behaviour, lower concentration (μM) increased the rate of leaf expansion but no beneficial effect was noticed at higher concentration (Haramaty and Leshem, 1997). Similarly, high concentration of NO (40-80 ppm) inhibited the growth of tomato, whereas, low concentration (0-20 ppm) stimulated the growth of tomato, lettuce (Hufton *et al.*,

1996) and pea seedlings (Haramaty and Leshem, 1997). NO also induced growth of root segments of maize as it was exerted by the treatment of indole acetic acid (Gouvea *et al.*, 1997). Moreover, SNP (0.1 mM) inhibited growth of hypocotyls in potato, lettuce and *Arabidopsis* (Beligni and Lamattina, 2000). Nevertheless, the root development was induced in cucumber by SNP (Pagnussat *et al.*, 2002). Exogenous application of NO inhibited the elongation of mesocotyl in maize seedlings (Zhang *et al.*, 2003). Contrary to this, increase in the leaf biomass of maize seedlings was observed by the endogenously produced and exogenously applied NO (An-Lizhe *et al.*, 2005). Treatment of low concentration of NO gas increased the shoot biomass (Jin *et al.*, 2009). Effect of NO on plant growth was found to be concentration dependent (Anderson and Mansfield, 1979; Gouvea *et al.*, 1997). Treatments of maize seedlings with lower concentration of SNP promoted root growth, whereas, higher concentration inhibited. Seedlings of canola raised from the seeds treated with lower concentration of SNP had more root length and dry mass, whereas, higher concentration reduced these parameters (Zanardo *et al.*, 2005). This dual behavior of NO donor SNP was also noted in wheat (Tian and Lei, 2006).

2.2.3.3 Effect of NO on senescence

Senescence is a process characterized by water loss and desiccation of plant tissues. Some reports suggested that NO have anti-senescence properties. Exogenous application of NO in pea leaves, under senescence promoting conditions decreased ethylene production (Haramaty and Leshem, 1997; Leshem *et al.*, 1998; Leshem 2000) due to the inhibition of ethylene biosynthesis. However, in *Arabidopsis* the level of ethylene enhanced significantly after exposure to NO gas (Magalhaes *et al.*, 2000). It was also observed that the NO emission decreased as ethylene emission increased from anthesis to senescence (Kopyra and Gwozdz, 2004). NO donors exert

a protective effect against ABA-induced senescence of rice leaves by diminishing ABA-dependent effect such as leaf senescence, enhanced H_2O_2 and melondialdehyde (MDA) content, reduction in GSH, ascorbic acid level and antioxidant enzyme activity (Huang and Kao, 2003). The protective effect was reversed by NO-scavenger (PTIO) suggesting that the observed phenomenon may be attributed to NO. Exogenous NO can protect naturally senescened soyabean cotyledons (Jasid *et al.*, 2009).

2.2.3.4 Effect of NO on NR activity

NR activity is one of the NO sources in plant roots (Jin *et al.*, 2009). Exogenous application of SNP (100 μM) significantly enhanced the activity of NR in leaves of maize plants (Zhang *et al.*, 2004) and in tomato plant (Jin *et al.*, 2009; Hayat *et al.*, 2010b), however, in case of pea and wheat root SNP did not influenced NR activity (Kolbert *et al.*, 2005).

2.2.3.5 Effect of NO on respiration

NO affects the mitochondrial functionality in plant cells and reduces total cell respiration due to strong inhibition of the cytochrome pathway. In carrot cell suspension, NO reduced total respiration by a half and this effect was accomplished by a significant increase of cell death. Similarly, in soybean cotyledon mitochondria the oxygen uptake was inhibited after NO treatment, but it was restored upon NO depletion (Millar and Day, 1996). It was concluded that alternative oxidase (AOX) may play a role in NO tolerance in higher plants. NO can also modulate another mitochondrial enzyme, tobacco aconitase, which is a constituent of Krebs cycle. Its inactivation by NO decreases the cellular energy metabolism which may results in reduced electron flow through the mitochondrial respiratory electron chain and

subsequent decrease in the generation of ROS, the natural byproduct of respiration (Navarre *et al.*, 2000; Giulivi *et al.*, 2006).

2.2.3.6 Effect of NO on stomatal movement

NO had also been reported to play a role in stomatal movement being, together with H_2O_2, an indispensable component of ABA-induced stomatal closure (Garcia-Mata and Lamattina, 2002, 2003; Desikan *et al.*, 2002, 2004). The exogenous application of NO to both monocot- and dicotyledonous epidermis strips induced stomatal closure, through a Ca^{2+} dependent process (Gracia-Matta and Lamattina, 2001). In *Pisum sativum* and *Vicia faba* plants, ABA induced increase in endogenous NO production was suggested as a reason for ABA induction of stomatal closure (Neill *et al.*, 2002). There are also some convergent evidence that support the involvement of NR through the production of NO in guard cell metabolism and stomatal movement (Gracia-Mata and Lamattina, 2002) leading to their closure (Neill *et al.*, 2002; Gracia-Mata and Lamattina, 2002; Neill *et al.*, 2009; Wilson *et al.*, 2009).

2.2.3.7 Effect of NO on chlorophyll content

The effect of NO on chlorophyll content was reported. NO donors (SNP) had been found to enhance chlorophyll content in potato, lettuce and *Arabidopsis* (Beligni and Lamattina, 2000). The role of NO in preserving and increasing chlorophyll content in pea and potato (Leshem *et al.*, 1998) was also proved. The protective effect of NO on the chlorophyll retention may reflect NO effects on iron availability. Strong evidence supporting a role of NO in the plant iron nutrition was presented by Graziano *et al.* (2002) as under iron-deficient growth conditions normally resulting in chlorosis. NO treatment increased the chlorophyll content in maize leaves upto the control level (Graziano *et al.*, 2002).

2.2.3.8 Effect of NO on photosynthesis

Photosynthesis is one of the most important physiological process in plant, whole metabolism of plant directly or indirectly depend on this process any change in photosynthetic rate automatically affected the rest of the processes in plant. However, the role of NO on photosynthesis is poorly understood, which is well indicated by the modest number of *in-vivo* and *in vitro* studies in this area with mixed results (Takashashi and Yamasaki, 2002; Yang *et al.*, 2004). Nitric oxide and its donors such as SNP, S-nitroso-N-acetylpenicillinamine, (SNAP), S-nitrosoglutathione (GSNO) had been found to regulate photosynthetic rate differentially (Hayat et al., 2010b). NO gas decreases net photosynthetic rate in *Avena sativa* and *Medicago sativa* leaves (Hill-Bennet, 1970). NO donor SNP had been found to decrease the enzymes of photosynthesis in wheat (Tu *et al.*, 2003) in *Phaseolus vulgaris* (Hayat *et al.*, 2005) and in *Pisum sativum* leaves (Wodala *et al.*, 2005, 2008).

NO is able to influence the photosynthetic electron transport chain directly. PS II is an important site of NO action (Wodala *et al.*, 2008). Within PS II complex, important binding sites of NO are the non-heme iron between Q_A and Q_B binding sites (Petrouleas and Diner, 1990), the Y_D, Tyr residue of the D2 protein (Sanakis *et al.*, 1997) and the manganese (Mn) cluster of the water-oxidizing complex (Schansker *et al.*, 2002).

NO donor SNAP does not modify the maximal quantum efficiency (Fv/Fm) but inhibits the linear electron transport rate, light induced pH formation (ΔpH) across thylakoid membrane, and decreased the rate of ATP synthesis (Takahashi and Yamasaki, 2002). Another NO donor SNP reduced Fv/Fm in the intact potato leaves but caused no difference in ΔpH dependent non-photochemical quenching (NPQ) (Yang *et al.*, 2004). A moderate decrease in Fv/Fm was also observed by SNP

treatment in pea leaves (Wodala, 2006). Moreover, NO donor had also been found to slow down the electron transfer between the primary and secondary quinone electron acceptor *in-vivo*, in a concentration dependent manner (Petrouleas and Diner, 1990; Wodala, 2006; Wodala *et al.*, 2008).

GSNO, another NO donor caused a significant decreased in Fv/Fm value in intact pea leaves and decreased steady-state qP, which indicated that NO increased the proportion of closed PSII reaction center (Wodala *et al.*, 2008), besides reducing steady state NPQ transiet (Wodala *et al.*, 2008) which resembles reaction center NPQ described by Finazzi *et al.* (2004) in *Hardeum vulgare*.

Wodala *et al.* (2008) suggested different chemical properties of NO donors and different experimental conditions jointly account for the above conflicting results.

2.2.3.9 Effect of NO on antioxidant system

It is now commonly accepted that NO act as a second messenger in plants. Now one of the most intriguing issues in NO biology is the dual function of this molecule as a potent oxidant and effective antioxidant (Beligni and Lamattina, 1999). This dual role of NO might depend on its concentration as well as on the status of the environment. Oxidative stress, is the common result of the action of many environmental stressing factors, manifests itself in a cell by an increased level of ROS (Mittler, 2002). The cytoprotective role of NO is mainly based on its ability to maintain the cellular redox homeostasis and regulate the level and toxicity of ROS.

The ability of NO to exert a protective function against oxidative stress caused by factors such as

(a) Reaction with lipid radicals, which stops the propagation of lipid oxidation.

(b) Scavenging the superoxide anion and formation of peroxynitrite ($ONOO^-$) which is toxic for plant but can be neutralized by ascorbate and glutathione.

(c) Activation of antioxidant enzymes (SOD, CAT, POX).

One of the fastest reaction of NO within biological system is its combination with superoxide anion (O_2^-) that lead to the formation of strong oxidant peroxinitrile ($ONOO^-$) (Wendehenne *et al.*, 2001; Neill *et al.*, 2003), the major toxic reactive nitrogen species (Stamler *et al.*, 1992). It exerts deleterious effects on DNA, lipids and proteins (Stamler *et al.*, 1992; Pryor and Squardrito, 1995; Yamasaki *et al.*, 1999). The exogenous application of NO stimulated the super-oxide dismutase (SOD) activity and/or diverts scavenging of the superoxide anion (Kopyra and Gwowdz, 2003) (Plate II).

The effect of NO on peroxidase is still scarce and somewhat controversial, depending on the concentration. The lower concentration of NO donor SNP had been found to increase peroxidase activity in *Brassica*, however, higher concentration proved inhibitory (Zanardo *et al.*, 2005). Similarly, earlier it was shown that ascorbate peroxidase activity was inhibited by higher SNP concentration in tobacco and canola (Clark *et al.*, 2000). Moreover, higher concentration of SNP had also been reported to inhibit coniferyl alcohol peroxidase activity in *Zinnia elegans* (Ferrer and Ros-Barcelo, 1999).

Treatment of wheat plant with lower concentration of SNP had been found to decrease the H_2O_2 content but antioxidant activity was enhanced (Tian and Lei, 2006, Hayat *et al.*, 2010b). NO production confers antioxidant protection in maize leaves (Tossi *et* al., 2009). Moreover, it had been reported that NO can react with lipid alcoxyl (LO^-) and peroxyl (LOO^-) radicals leading to the expectation that NO could

stop the propagation of radical mediated lipid oxidation in a direct fashion (Lamotte *et al.*, 2004; Tian and Lei, 2006). NO decreased TBARS content in wheat seedlings (Tian and Lei, 2006).

2.2.3.10 Effect of NO on programmed cell death (PCD)

There are numerous and often contradictory reports concerning NO and programmed cell death (PCD). It had been reported that elevated levels of NO was sufficient to induce cell death in *Arabidopsis* cell suspension, independently from ROS (Clarke *et al.*, 2000). The influence of NO and ROS donors on PCD was investigated in tobacco (de pinto *et al.*, 2002). The increase of either NO or ROS separately did not induce cell death, whereas, the simultaneous increase of NO and ROS activated a process of cell death with typical cytological and biochemical features of PCD (de Pinto *et al.*, 2002). Moreover, the interaction between NO and ROS in PCD induction was also investigated in soybean cell suspension (Delledonne *et al.*, 2001). Contrary to that in *Taxus brevifolia* and *Kalanchoe diagremontianna*, SNP caused NO burst, which proceeded to a significant increase in nuclear DNA fragmentation and cell death (Pedroso *et al.*, 2000). On the other hand, it was suggested that NO dependent delay of development PCD induced by gibberelic acid (GA) in barley, aleurone layer. Although NO is able to inhibit PCD in GA treated cells, it does not have a general effect on cellular metabolism and is only predicted as a specific endogenous modular of PCD (Beligni and Lamattina, 2002).

2.2.4 NO and cross-talk with classical plant hormones

Under this heading we discussed the cross talk between NO and other plant hormones that act simultaneously on different physiological and biochemical process in plant.

2.2.4.1 Auxins and NO

NO induced the elongation of maize root segments in a dose-dependent manner (Gouvea *et al.*, 1997), it has been proposed that the auxin, indole acetic acid (IAA) and NO might share some common steps in the signal transduction pathway, because both elicit the same responses in plant. The dependence of auxin on NO, in the induction of adventitious root development was recently demonstrated in cucumber explants (Pagnussat *et al.*, 2002). Moreover, explants from wood species were also responsive to NO treatment to induce adventitious root formation (Lamattina *et al.*, 2001).

In cucumber explants, IAA treatment induces a transient increase in the level of endogenous NO in the basal region of the hypocotyl, where the new meristems develop (Pagnussat *et al.*, 2002). This localized NO bulk might stimulate the GC-catalyzed synthesis of cGMP (McDonald and Murad, 1995). The GC inhibitor reduced adventitious root formation both in IAA and NO treated cucumber explants. This effect was reversed when permeable cGMP analog was added together with GC inhibitor and NO or IAA (Pagnnussat *et al.*, 2004). Earlier, in tobacco activation of defense genes by NO was also induced by cGMP (Durner *et al.*, 1998). cGMP can act via cADPR which in turn, regulates Ca^{2-} levels as was reported in various plant systems (Leckie, 1998). NO can also act via cGMP-independent pathway, activating phosphatases and protein kinases including MAPKs. Interestingly, a rapid and transient increase of MAPK activity in response to low level of auxins was reported in *Arabidopsis* seedling roots (Pfeiffer *et al.*, 1994). IAA-induced endogenous NO bulk in roots could result in a bifurcated signal transduction pathway in which NO mediates a cGMP-dependent or independent increase of cytosolic Ca^{2+}, which in turn triggers changes in plant gene expression.

2.2.4.2 ABA and NO

ABA regulates various vital processes in plant. Stomatal movement is one of them. In the guard cell, ABA induces the depolarization of the plasma membrane potential that leads to the generation of a driving force for K^+ efflux, inactivates K^+ influx through inward-rectifying K^+ (K^+_{in}) channels, and activates a current through outward-rectifying (K^+_{out}) channels. These changes together with both slow and fast activating anion channels, facilitates the net loss of salt from the cell (Blatt, 2000). Both cytosolic free Ca^{2+} concentration (Ca^{2-}_{cyt}) and cytosolic pH have been reported to participate as second messengers of this response (Blatt and Grabor, 1997; Sokolovski *et al.*, 2005). ABA induces guard cell $[Ca^{2+}]_{Cyt}$ elevation either by influx from extra cellular space or by release from internal source (Suhita *et al.*, 2004), that lead to the loss of guard cell turgor, leading to stomatal closing.

On the other hand exogenous application of NO to both monocot and dicotyledonous epidermis strips was sufficient to induce stomatal closure, through a Ca^{2+}-dependent process (Gracia-Mata and Lamattina, 2001). Moreover, it was further reported that in *Pisum sativum* and *Vicia faba*, ABA induces an increase of endogenous NO levels. This bulk of ABA-induced NO production was reported to be sufficient and necessary for ABA induction of stomatal closure (Garcia-Mata and Lamattina, 2002; Neill *et al.*, 2002; Saito *et al.*, 2009). The participation of NO as a signal molecule in guard cell movement is a very recent topic; much work has still to be done in this field.

2.2.4.3 Cytokinins, Gibberellins, NO

Cytokinins (CKs) in particular can stimulate photomorphogenic responses, mainly those related with the deterioration process and pigment synthesis (Thomas *et al.*, 1997). In dark grown seedlings, exogenous application of CK inhibits hypocotyl

elongation (Chang *et al.*, 1999). Likewise, NO significantly reduce hypocotyl elongation in *Arabidopsis* and lettuce seedlings grown in the dark (Belgini and Lamattina, 2000). In cotyledons and leaves growing under dark condition, CK treatment cannot cause etiolation to revert completely. Moreover, NO is also able to slightly increase the chlorophyll level in wheat seedlings grown in dark, but the NO effect is strongly potentiated by the short term light pulse (Belgini and Lamattina, 2000). Thus, the NO effect is similar to that of CKs and probably some components are induced only via phytochrome to allow complete greening. Moreover it had been demonstrated that the heterotrimeric G-protein, cGMP, and calmodulin stimulated biological processes are mediated by phytochrome (Bowler *et al.*, 1994).

Some seeds are dependent on light for germination under certain conditions. Seeds of lettuce also found to be phytochrome dependent, and it was demonstrated that NO donors are able to promote germination in the dark to the same extent as both as GA treatment or a 5 min pulse of white light (Belgini and Lamattina, 2000). GA was observed to act like the active form of phytochrome to induce germination (Yang *et al.*, 1995). However, seeds were able to germinate in the light, in the presence of the NO scavenger cPTIO suggesting that light and NO can stimulate germination in different ways (Belgini and Lamattina, 2000). Whether, GA and NO act in promoting germination through the same or different pathway remain to be determined. NO and GA promote germination in tomato (Piterkova *et al.*, 2009) under osmotic stress.

2.2.4.4 Ethylene and NO

Ethylene plays an active role in many plant responses (Abeles *et al.*, 1992). It was suggested that NO and ethylene cause an antagonistic effect during maturation and senescence of the plant (Leshem and Pinchasov, 2002). It was demonstrated that endogenous NO and ethylene content maintain an inverse correlation during the

ripening of strawberries and avocados (Leshem and Pinchasov, 2002), and bananas (Cheng *et al.*, 2009) while unripe, green fruits contain high NO and low ethylene concentration; the maturation process is accompanied by a marked decrease of NO concomitant with an increase of ethylene (Leshem and Pinchasov, 2002).

2.3 BRASSINOSTEROIDS

Growth is an organized, well-coordinated complex process where metabolism provides the energy and the building blocks. However, it is the relative hormone level that regulates the pace of growth of each individual plant part, to produce a form that is recognized as a plant (Plate III). Earlier, only five groups of hormones (auxins, gibberellins, cytokinins, abscissic acid and ethylene) were designated as regulators of plant growth. However, in the recent past, compelling evidences have been put forward to classify an additional group of steroidal substances (brassinosteroids), first isolated from rape (*Brassica napus* L.) pollen, as a new class of phytohormones.

It was in 1970, when Mitchell and co-workers screened the pollens of nearly sixty species, out of which the extract from about thirty species generated growth in bean seedlings. This growth promoting substance was called "brassin". The search for its active factor(s) was collectively approached in 1974 by the USDA scientists working at northern regional research centre (NRRC), Peoria; Eastern Regional Research Centre (ERRC), Philadelphia and Beltsville Agricultural Research Centre (BARC), Maryland. Bee-collected pollens (500 lb) were processed through a pilot plant-size solvents (2-propanol) extraction procedure at ERRC and succeeded in partial purification at BARC. However, it was crystallized at NRRC and was subjected to x-ray analysis to establish its structure. This biologically active plant growth promoter was found to be steroidal lactone ($C_{28}H_{48}O_6$) and was named as "brassinolide" (BL) which was later renamed as "brassinosteroid". All natural

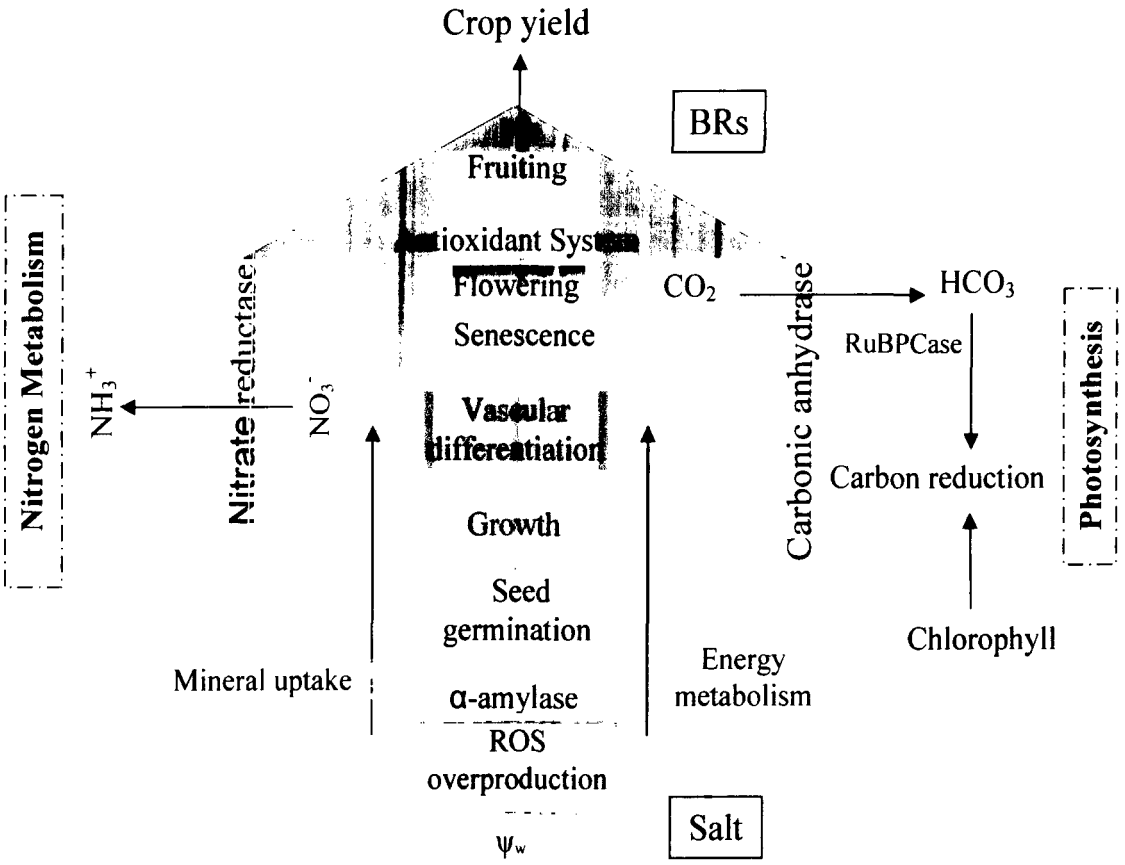


Plate-III. Salt stress affects a wide array of physiological processes viz. nitrogen, carbon and energy metabolism mineral uptake and generation of ROS where BR application counteracts almost all aspects depending upon dose interaction of stressor and BR.

brassinosteroids have a common 5-choleston skeleton and its structural variants come from the type and the orientation of functionalities on the skeleton. Their low level in plants is not uniform throughout its body but young growing tissues have comparatively a larger share than the mature tissues (Yokota and Takahashi, 1986). The richest sources are pollen and immature seeds where its concentration ranges between 1-100 ng g^{-1} fresh mass, whereas, shoots and leaves have about 0.01-0.1 ng g^{-1} fresh mass (Takatsuto, 1994). Till now more than 70 brassinosteroids, structurally and functionally different form each other, have been characterized (Hayat and Ahmad, 2003d). Out of which, three (BL, 24-epiBL and 28-homoBL) are being largely applied to have an economical impact on plant metabolism, growth and productivity.

2.3.1 Effect of brassinosteroids on seed germination

Endogenous BRs have been identified in the seeds of several species, including pea (Yokota and Takahashi, 1986), *Arabidopsis thaliana* (Schmidt *et al.*, 1997) and *Lychnis viscaria* (Friebe *et al.*, 1999). It is well documented that brassinosteroids promote seed germination, like other hormones. The treatment of the seeds of *Lepidium sativus* (Jones-Held *et al.*, 1996) and *Eucalyptus camaldulensis* (Sasse *et al.*, 1995) with BL improved percent germination. Similarly brassinosteroids promoted seed germination in case of *Brassica napus* (Chang and Cai, 1998), wheat (Sairam *et al.*, 1996; Hayat *et al.*, 2003a), tomato (Vardhini and Rao, 2000), tobacco (Leubner-Metzger, 2001), barley (Kartal *et al.*, 2009) and *Brassica jucnea* (Sirhindi *et al.*, 2009), Moreover, BL, 24-epiBL and 28-homoBL promoted seed germination in groundnut (Vardhini and Rao, 1997). BR application has been reported to enhance the germination of certain parasitic angiosperms (Takeuchi *et al.*, 1991, 1995), cereals (Gregory, 1981; Yamaguchi *et al.*, 1987), *Arabidopsis* (Steber and McCourt, 2001).

Pretreatment with BL stimulated the germination and seedling emergence of aged rice grains (Yamaguchi *et al.*, 1987) and seed treatment of barley accelerated subsequent seedling growth (Gregory, 1981). It is, however, not certain whether the promoting effect of BR in cereal grains is actually manifested only at the level of seedling growth and/or also at the level of germination *per se*.

In *A. thaliana*, BR promotes the germination of pre-chilled (i.e. non-dormant) seeds of BR-deficient biosynthesis mutant *det2-1* and the BR-insensitive response mutant *bri1-1* imbibed in the light (Steber and McCourt, 2001; Zang *et al.*, 2009). Seed germination of *det2-1* and *bri1-1* is more strongly inhibited by ABA than the wild type and BR is, able to partially overcome the inhibition of germination by ABA (Finkelstein *et al.*, 2008; Zhang *et al.*, 2009; Xue *et al.*, 2009). BR treatment rescues the germination phenotype of the severe GA-deficient biosynthesis mutant *ga1-3*, which normally requires GA treatment for dormancy release and germination. BR treatment also partially rescues the germination phenotype of the severe GA-insensitive response mutant *sly1* (*sleepy1*), which cannot be rescued by treatment with GA. Interestingly, a new allele for *sly1* was identified in a screen for BR-dependent germination and also proposed an interaction between BR and GA signaling in seeds (Steber *et al.*, 1998; Steber and McCourt, 2001). This is further supported by the germination phenotype of the *gpa1* mutant of *Arabidopsis* (Ullah *et al.*, 2002). BR promotes seedling elongation and germination of non-photodormant tobacco seeds, but do not appreciably affect testa rupture and subsequent induction of ßGlu I in the micropylar endosperm (Leubner-Metzger, 2001, 2003). Treatment with BR accelerates endosperm rupture of tobacco seeds imbibed in the light. Promotion of endosperm rupture by BR is dose-dependent and 0.01 µM BL is most effective.

2.3.2 Effect of brassinosteroids on flowering

There has been very limited use of steroids in regulating flowering. The number of flowers in strawberry increased by the application of BRs at the foliage (Pipattanawong *et al.*, 1996). However, in case of grapes, the application of brassinosteroids in autumn improved the number of flowers but inhibited if the time of application is delayed to late winter (Rao *et al.*, 2002). BR regulated flowering in *Arabidopsis* (Clouse, 2008; Yu *et al.*, 2008).

2.3.3 Effect of brassinosteroids on senescence

It is the process, which refers to endogenously regulated deteriorative changes that become the natural cause of death of cells, tissues, organs or that of the whole organism (Arteca, 1997). Like other hormones (Rao *et al.*, 2002), BRs also play a crucial role in regulating the processes leading to senescence. The BL promotes senescence in *Xanthium* and *Rumex* explants (Mandava *et al.*, 1981). In addition to it, BRs also accelerate senescence in the detached cotyledons of cucumber seedlings (Zhao *et al.*, 1990) and leaves of mung bean seedlings (He *et al.*, 1996). However, BR deficient *Arabidopsis* mutants exhibited delayed senescence of chloroplast (Li *et al.*, 1996). Similarly, the senescence of the leaves of mungbean and mustard was delayed, if supplied with 28-homoBL at early stage of growth (Fariduddin, 2002). During a search of senescence associated genes, He *et al.* (2001) developed a preliminary model for leaf senescence regulating network in *Arabidopsis*, where signals such as ABA, jasmonic acid, ethylene, darkness, dehydration and aging activated 147 senescence associated enhancer trap line. 24-epiBL could activate some of these but associated genes have not yet been cloned.

2.3.4 Effect of brassinosteroids on photosynthesis

The aqueous solution of 28-homoBL, applied to the foliage of wheat and mustard (Sairam, 1994; Hayat *et al.*, 2000; 2001a) and geranium (Swamy and Rao, 2009), *Cucumis sativus* (Xia *et al.*, 2009), mungbean (Ali *et al.*, 2008a) or applied as seed soaking treatment to mungbean (Fariduddin *et al.*, 2003; 2004) and dialkylaminoethylalkanoate or epiBL, in association with GA_3, to spinach enhanced the photosynthetic rate (Liang *et al.*, 1998). Foliar spray of aqueous solution of BR to wheat and mustard (Braun and Wild, 1984), epibrasisnolide to seedlings of cucumber (Ding *et al.*, 1995), brasisnolide to rice (Fujii *et al.*, 1991) and *Vicia faba* (Pinol and Simon, 2009) increased the rate of CO_2 assimilation. Likewise, the foliar application of 24-epiBL enhanced the light saturated net CO_2 assimilation rate and carboxylation rate of rubisco, thereby increasing the capacity of CO_2 assimilation in the Calvin cycle (Yu *et al.*, 2004; Xia *et al.*, 2009). However, the epicotyl of cucumber, did not respond to epiBL but the transport of the labeled (^{14}C) glucose towards the epicotyl was favoured (Nakajima and Toyama, 1995). Similarly, Hill activity in the foliage of *Vigna radiata* was favourably affected on being supplemented with aqueous solution of 28-homoBL (Bhatia and Kaur, 1997).

2.3.5 Effect of brassinosteroids on chlorophyll

The total chlorophyll content or its fractions increased in the leaves of *Vigna radiata* (Bhatia and Kaur, 1997) and *Brassica juncea* (Hayat *et al.*, 2001a) by 28-homoBL and in *Cucumis sativus* (Yu *et al.*, 2004) and *Vicia faba* (Pinol and Simon, 2009) by epiBL, applied directly to their foliage. Similarly, the values for the above parameters increased in the leaves of rice (Wang, 1997), *Cicer arietinum* (Fariduddin *et al.*, 2000), *Brassica juncea* (Hayat and Ahmad, 2003b). *Vigna radiata* (Fariduddin

et al., 2003) and *Pelargonium graveolens* (Swamy and Rao, 2009) raised from the seeds given pre sowing treatment with BRs.

2.3.6 Effect of brassinosteroids on carbonic anhydrase activity

Carbonic anhydrase (CA) is the second most abundant soluble protein, other than RuBPcase, in C_3-chloroplast (Reed and Graham, 1981; Okabe *et al.*, 1984). It is a zinc containing protein with a molecular weight of 180 KDa (Lawlor, 1987) and is ubiquitous enzyme, among living organisms. It catalyzes the reversible inter conversion of bicarbonates (HCO_3^-) and CO_2 (Sultemeyer *et al.*, 1993). The rate of conversion of HCO_3^- to CO_2 is normally slow in alkaline conditions. However, CA activates the use of HCO_3^- in the production of CO_2 (Lawlor, 1987). In C_3 plants, CA has a close association with RuBPCase where it elevates the level of CO_2 at its active site (Badger and Price, 1994). An increase in the activity of CA in the leaves was attained by the application of 28-homoBL to the shoot of the *Brassica juncea* (Hayat *et al.*, 2000, 2001a). Moreover, the seedlings of wheat and mungbean, raised from the grains treated with 28-homoBL, possessed high CA activity in their leaves (Hayat *et al.*, 2001b; Fariduddin *et al.*, 2003). Seed application of EBL reduced the toxic effect of cadmium on carbonic anhydrase activity (Anuradha and Rao, 2009).

2.3.7 Effect of brassinosteroids on nitrate reductase activity

The process of reduction of nitrate is catalyzed by the enzyme, nitrate reductase (NR), the level of which increased in the plants of rice (Mai *et al.*, 1989), maize (Shen *et al.*, 1990), water stressed wheat (Sairam, 1994), *Lens culinaris* (Hayat and Ahmad, 2003 a, b), *Vigna radiata* (Fariduddin *et al.*, 2004) and wheat (Hayat *et al.*, 2001b), and in the seeds of wheat (Hayat and Ahmad, 2003c) by the application of BRs.

2.3.8 Effect of brassinosteroids on the vascular tissue

The first report of a role for BRs in the differentiation of vascular tissues came in 1991 (Clouse and Zurek, 1991). Jerusalem artichoke (*Helianthus tuberosus)* cells transferred to the xylem differentiation medium, in the presence of auxin and cytokinins will differentiate into xylem elements in 72 - 96 hours. Very few vascular elements develop in the first 24 hours following transfer into this medium. However, nanomolar concentrations of BL included in the medium, resulted in a 10-fold increase in xylem differentiation, this was observed in the first 24 hours. Also significant increases in cell numbers were observed, indicating a role for BRs in cell division and differentiation (Clouse and Zurek, 1991).

Zinnia elegans has been used extensively to study the formation of xylem/tracheary elements, a process that has three distinct stages (Fukuda, 1997). BRs have been implicated in the transition between Stage II and Stage III where secondary wall formation and cell death occurs (Fukuda, 1997). It has previously been shown that the effects of Uniconazole (a putative BR biosynthesis inhibitor) prevent differentiation of *Zinnia* mesophyll cells into tracheary elements and this inhibition was overcome by exogenous BR application (Iwasaki and Shibaoka, 1991). Uniconazole appears to suppress the transcription of genes involved in the final stages of differentiation but could be recovered by the exogenous application of BL (Yamamoto *et al.,* 1997). This suggests that BRs are synthesized immediately prior to secondary cell wall development and cell death and possibly induces entry into this stage (Yamamoto *et al.,* 1997).

2.3.9 Effect of brassinosteroids on stressed plants

Plants respond to abiotic and biotic factors in environment. These include heavy metal action, wounding, drought, high salt concentration, changes in

temperature and light, and pathogen and pest attack. Abiotic stress leads to morphological, physiological, biochemical and molecular changes. Drought, salinity, extreme temperatures and oxidative stress are often interconnected, and may induce similar cellular damage. For example, drought and/or salinization are manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cells. Oxidative stress, which frequently accompanies high temperature, salinity, or drought stress, may cause denaturation of functional and structural proteins. As a consequence, these diverse environmental stresses often activate similar cell signaling pathways and cellular responses, such as the production of stress proteins, up regulation of antioxidants and accumulation of compatible solutes. Biochemical adaptation in plants involves various changes in the biochemistry of the cell. These changes include the evolution of new metabolic pathways, the accumulation of low molecular weight metabolites, the synthesis of special proteins, detoxification mechanisms and changes in phytohormone levels (Irfan *et al.*, 2010). Adaptation represents the ability of a living organism to fit into a changing environment, at the same time improving its chances of survival and reproducing itself.

2.3.9.1 Response of brassinosteroids to saline stress

The application of 24-epiBL resulted in substantial improvement in seed germination and seedling growth of *Zea mays* under saline stress (Arora *et al.*, 2008). Seed germination in the presence of 150 mM NaCl was enhanced by 24-epiBL, but when seedlings were grown hydroponically in salt, uptake of 24-epiBL through roots caused more damage (Sasse *et al.*, 1995). BRs removed the salinity-induced inhibition of seed germination and seedling growth in case of *Oryza sativa*. BRs also restored the level of chlorophyll and increased NR activity under salt stress. The activity of

this enzyme plays a pivotal role in the supply of nitrogen and the growth and productivity of plants, especially in cereals. The reduced NR activity in the leaves of salt-stressed plants is attributed to salinity inhibited nitrate transport to the shoot, which in turn is due to interference with nitrate uptake and xylem loading (Anuradha and Rao, 2003). Likewise, seed soaking of 28-homoBL enhanced the nitrogen fixing capability in chickpea plants pre-treated with NaCl (Ali *et al.,* 2007). The plants also showed increased dry matter accumulation, together with an increase in the activities of NR and CA. 28-homoBL treated plants also possessed higher seed yield in comparison to the plants subjected to NaCl stress, at harvest. Similarly, the spray of 28-homoBL to the foliage or supplied through roots of *Brassica juncea* plants, generated from the seeds soaked in NaCl, enhanced growth, nucleic acid content, ethylene production and seed yield (Hayat et al., 2006; 2007a, b). Wheat germ agglutinin (WGA), a classic cereal lectin, level is increased in plants under unfavourable conditions: fungal infection, drought and osmotic stress, salinity and hyperthermia. ABA is involved in the control of WGA synthesis and accumulation. It was demonstrated that BRs did not influence the ABA content but enhanced only the accumulation of lectin under salt stress in the roots of *Triticum aestivum*. This indicates the possibility of ABA-independent control of the lectin content by BRs. Salt treatment resulted in rapid ABA accumulation, which occurred prior to 5-fold increase in the level of lectin. On the other hand, combined treatment BRs with NaCl resulted in partial growth recovery as compared to NaCl alone. BRs reduced the salinity-induced accumulation of BRs and WGA in roots by 50%. Possibly BRs could be involved in the hormonal control of WGA level along with ABA. BRs evidently exert a protective action on wheat seedlings via a decrease in the salt-induced ABA

and WGA accumulation in roots (Shakirova and Bezrukova, 1998; Shakirova *et al.*, 2002).

2.3.10 Effect of brassinosteroids on yield of crops

Once the presence of BRs in plants was established, the next phase was to explore the possibilities of using these new substances in improving the yield of economically useful plants. Meudt and his associates (1983, 1984) used BL to improve the yield of lettuce, radish, bush bean and pepper. Foliar application of dilute aqueous solution of BL improved the yield in wheat and mustard (Braun and Wild, 1984), rice, corn and tobacco (Yokota and Takahashi, 1986). BRs were also found to increase the growth and yield of sugarbeet (Schilling *et al.*, 1991), legumes (Kamuro and Takatsuto, 1991) and rape seed (Takematsu and Takeuchi, 1989; Hayat *et al.*, 2000; 2001b). Application of 28-homoBL and 24-epiBL significantly increased yields of potato, mustard, rice and cotton (Ramraj *et al.*, 1997), *Lens culinaris* (Hayat and Ahmad, 2003a, b), *Vigna radiata* (Fariduddin *et al.*, 2003) and that of corn, tobacco, watermelon, cucumber and grape (Ikekawa and Zhao, 1991) respectively. Foliar application of BL, 24-epiBL (Vardhini and Rao, 1997) and 28-homoBL (Vardhini and Rao, 1998) was highly effective in enhancing the yields of groundnut and tomato (Vardhini and Rao, 2001). Moreover, in China, 28-homoBL has been registered as a plant growth regulator in case of tobacco, sugarcane, rapeseed and tea.

2.3.11 Future prospects of brassinosteroids

Forty years of research on BRs has brought into light several vital functions of this class of phytohormones in the regulation of plant growth, development and productivity. Further progress in the investigation of mechanism of BRs action in plants, on one hand, and elaboration of economically feasible schemes of synthesis of natural BRs and their analogs, on the other hand, will surely make a basis for the

inclusion of this new class of plant hormones in the regular package of chemicals used for optimizing agricultural production. Hopefully, as the research will progress, much more knowledge will be added to the present literature. It has been stated earlier that the application of these steroids to plants generates varied physio-morphological changes by involving the genome and also do not initiate co-evolution of pests, enriching our arsenal of plant protection strategies, in the twenty first century. Moreover, the knowledge of the physical and chemical properties of these steroids is tempting us to consider them highly promising, environment friendly and promoter of agricultural productivity. One of the major constraints, to employ brassinosteroids at larger scale, in the fields is their high cost. However, recent progress, in chemical synthesis of BRs and their analogues has led us to economically feasible approaches that has brought large scale application very near to the reach of the farmers. Pesticidal companies in China and Japan have started synthesizing brassinosteroids, on a commercial scale. In India, also Godrej Agrovet Ltd., Mumbai, introduced 28-homoBL in the market. We predict a better future for brassinosteroids in realizing crop yields, during the 21st century.

2.4 Concluding remarks

The available literature reviewed above revels that salinity is toxic to plants that retards growth, metabolism and yield of plants. On the other hand BRs has been emerged out as a new phytohormone and NO, a new signalling molecule, that had a promoting effect on the general physiology of plants, but not much attention has been given to the impact of BRs and NO on the salinity stressed plants. Therefore, this study was planned to observe the effect of BRs and NO on the salinity induced changes in *Lycopersicon esculentum.*

Contents

CHAPTER - 3

MATERIALS AND METHODS

Seeds of tomato (*Lycopersicon esculentum*) var. K-21 were used as a test material. Eight pot experiments were conducted during 2007-09, to study the effect of brassinosteroids (28-homobrassinolide and 24-epibrassinolide) and nitric oxide (sodium nitroprusside) on tomato plants grown under different level of salinity (NaCl) stress at selected stages of growth. The comprehensive details of the material used and the methodologies adopted, during the course of the present investigation are presented in this chapter.

3.1 Seeds

The authentic seeds of *Lycopersicon esculentum* var. K-21 were obtained from National Seed Corporation Ltd. New Delhi, India. Before the start of each experiment, the healthy seeds of uniform size were tested for their per cent viability. Mercuric chloride solution (0.01%) was used for surface sterilization of seeds. This was followed by rinsing the seeds with double distilled water (DDW), at least thrice, to remove the adhering solution of the mercuric chloride.

3.2 Preparation of pots

Each earthen pot (25 x 25 cm) was filled with sandy loam soil mixed with farmyard manure in the ratio of 9:1. The pots were amended with recommended dose of fertilizers. The pots were arranged in a simple randomized block design in a net house.

3.3 Hormone and their preparation

28-homobrassinolide (HBL) and 24-epibrassinolide (EBL) were obtained from Godrej Agrovet Ltd., Mumbai, India and Sigma Chemicals, St. Louis, USA,

respectively. Stock solution (10^{-4}M) was prepared by dissolving required quantity of hormone in 5 ml of ethanol, in 100 ml volumetric flasks. Five ml surfactant "Tween-20" was added to it and final volume was made upto the mark by using DDW. The desired concentrations of HBL or EBL were prepared by the dilution of stock solution.

3.4 Source of nitric oxide (NO)

Sodium nitroprusside (SNP) was used as the source of NO. The required concentrations of SNP were prepared by dissolving the requisite amount in DDW.

3.5 Experiment 1

This experiment was laid down according to simple randomized design during the winter season (September-February) 2007-08. The surface sterilized seeds of *Lycopersicon esculenium* var. K-21 were treated in the following manner:

A. The seeds were soaked in the solution of NaCl (50, 100 or 150 mM) for 8 hours (duration of soaking were based on the preliminary trial).

B. The seeds were soaked in DDW for 8 hours to be used as control.

The treated seeds were sown in earthen pots to create the nursery. At 20 days after sowing (DAS) these treated seedlings were subsequently transplanted to the maintained pots, under similar conditions as in the case of nursery pots. Each treatment was replicated thrice. Irrigation was done with tap water as and when required. The plants were sampled at 45 and 60 DAS to study the following parameters.

1. Length of root and shoot per plant
2. Fresh and dry mass of root and shoot per plant
3. Leaf area

4. Relative water content (RWC)
5. SPAD chlorophyll
6. Photosynthetic rate (P_N)
7. Stomatal conductance (g_s)
8. Internal CO_2 concentration (C_i)
9. Water use efficiency (WUE)
10. Transpiration rate (E)
11. Leaf nitrate reductase (NR) activity
12. Leaf carbonic anhydrase (CA) activity
13. Leaf peroxidase (POX) activity
14. Leaf catalase (CAT) activity
15. Leaf superoxide dismutase (SOD) activity
16. Leaf proline content

3.6 Experiment 2

This experiment was laid down according to simple randomized design, during the winter season (September – February) of 2007-08. The surface sterilized seeds of *Lycopersicon esculentum* var. K-21 were treated in the following manner to create the nursery:

i) Seeds were sown in the pots containing 0 mg of NaCl/Kg of soil

ii) Seeds were sown in the pots containing 2.9 mg of NaCl/Kg of soil

iii) Seeds were sown in the pots containing 5.8 mg of NaCl/Kg of soil

iv) Seeds were sown in the pots containing 8.7 mg of NaCl/Kg of soil

At 20 DAS there treated seedling were subsequently transplanted to the maintained pots. Each treatment was replicated thrice. Irrigation was done with tap

water as and when required. The plants were sampled at 45 and 60 DAS and the characteristics studied were same as in experiment 1.

3.7 Experiment 3

This experiment was laid down according to simple randomized design during the winter season (September - February) of 2007-08. The surface sterilized seeds of *Lycopersicon esculentum* variety K-21 were treated in the following manner:

A. The seeds were soaked in the solution of SNP (10^{-4}M, 10^{-5}M or 10^{-6}M) for 8 hours (duration of soaking and concentration of SNP were based on the preliminary trial).

B. The seeds were soaked in DDW for 8 hours to be used as control.

The treated seeds were sown in earthen pots to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots, under similar conditions as in case of nursery pots. Each treatment was replicated thrice. Irrigation was done with tap water as and when required. The plants were sampled at 45 and 60 DAS and the characteristics studied were same as in experiment 1.

3.8 Experiment 4

This experiment was conducted according to simple randomized design during the winter season (September - February) of 2007-08. The surface sterilized seeds of *Lycopersicon esculentum* variety K-21 were sown in earthen pots to create nursery. At 20 DAS, these seedlings were subsequently transplanted to the maintained pots. These seedlings were treated as follows:

a) The foliage of 44 day old seedlings was sprayed with DDW, to be used as control.

b) The foliage of 44 day old seedlings was sprayed with aqueous solution of HBL (10^{-6}M, 10^{-8}M or 10^{-10}M).

c) The foliage of 44 day old seedlings was sprayed with aqueous solution of EBL (10^{-6}M, 10^{-8}M or 10^{-10}M).

Irrigation was done with tap water as and when required. The plants were sampled at 45 and 60 DAS and the characteristics studied were same as in experiment 1.

3.9 Experiment 5

This experiment was laid down according to simple randomized design, during the winter season (September - February) of 2008-09. The surface sterilized seeds of tomato (var. K-21) were soaked in NaCl (50 mM, 100 mM or 150 mM) or DDW for 8 hours and these seeds were sown in earthen pots to create the nursery. At 20 DAS, these seedlings were subsequently transplanted to the maintained pots. These seedlings were treated as follows:

a) The foliage of the 44 day old seedlings raised from the seeds soaked in DDW for 8 hours was sprayed with water.

b) The foliage of 44 day old seedlings raised from the seeds soaked in 50 mM of NaCl for 8 hours was sprayed with 10^{-8}M of aqueous solution of HBL or EBL. The concentration of HBL or EBL was selected on the basis of the experiment 4.

c) The foliage of 44 day old seedlings raised from the seeds soaked in 100 mM of NaCl for 8 hours was sprayed with 10^{-8}M of aqueous solution of HBL or EBL.

d) The foliage of 44 day old seedlings raised from the seeds soaked in 150 mM of NaCl for 8 hours was sprayed with 10^{-8}M of aqueous solution of HBL or EBL.

Irrigation was done with tap water as and when required. The plants were sampled at 45 and 60 DAS and the characteristics studied as well as the other experimental conditions were same as that of experiment 1.

3.10 Experiment 6

This experiment was laid down according to simple randomized block design during the winter season (September-February) of 2008-09. The surface sterilized seeds of tomato (*Lycopersicon esculentum* var. K-21) were sown in pots containing 2.9, 5.8 or 8.7 mg of NaCl per Kg of soil to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. These seedlings were treated as follows:

a) The foliage of the 44 day old seedlings raised from the seeds sown in pots was sprayed with DDW to be used as control.

b) The foliage of the 44 day old seedlings raised from the seeds sown in the pots containing 2.9 mg NaCl/Kg of soil was sprayed with aqueous solution of 10^{-8}M of HBL or EBL.

c) The foliage of the 44 day old seedlings raised from the seeds sown in the pots containing 5.8 mg NaCl/Kg of soil was sprayed with aqueous solution of 10^{-8}M of HBL or EBL.

d) The foliage of the 44 day old seedlings raised from the seeds sown in the pots containing 8.7 mg NaCl/Kg of soil was sprayed with aqueous solution of 10^{-8}M of HBL or EBL.

Each treatment was replicated thrice. Irrigation was done with tap water as and when required. These plants were sampled at 45 and 60 DAS to study the characteristics mentioned in experiment 1. All the other experimental conditions were same as in experiment 1.

3.11 Experiment 7

This experiment was conducted according to simple randomized design during the winter season (September–February) of 2008-09. The seeds of *Lycopersicon esculentum* var. K-21 were surface sterilized with the mercuric chloride solution and subsequently soaked in the solution of NaCl (50, 100 or 150 mM) for 8 hours and than transferred to the solution of 10^{-5}M of SNP for 8 hours again (the concentration of SNP was based on experiment 3). Another set of seeds were soaked in DDW for 16 hours to be used as control. These seeds were sown in the pots to create nursery. After 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. Each treatment was replicated thrice. Irrigation was done with tap water as and when required. The plants were sampled at 45 and 60 DAS to study the characteristics mentioned in experiment 1. All the other experimental conditions were same as in experiment 1.

3.12 Experiment 8

This experiment was laid down according to simple randomized design during the winter season (September–February) of 2008-09. The surface sterilized seeds of tomato (*Lycopersicon esculentum* var. K-21) were soaked in SNP (10^{-5}M) for 8 hours and sown in the pots in the following manner:

a) The seeds were sown in the pots containing 2.9 mg NaCl/Kg of soil

b) The seeds were sown in the pots containing 5.8 mg NaCl/Kg of soil

c) The seeds were sown in the pots containing 8.7 mg NaCl/Kg of soil

For the control set seeds were soaked in DDW for 8 hours and sown in the pots containing 0 mg NaCl/Kg of soil.

At 20 DAS the seedlings obtained from these treatments were subsequently transplanted to the maintained pots. Each treatment was replicated thrice. Irrigation was done with tap water as and when required. The plants were sampled at 45 and 60 DAS and the characteristics studied were same as in experiment 1. All the other experimental conditions were same as in experiment 1.

3.13 Parameters studied

The methods applied to assess various growth, biochemical and enzymatic parameters are described below:

3.13.1 Growth parameters

The following methods were adopted to assess the various growth parameters.

3.13.1.1 Shoot and root length per plant

One plant from each pot was taken and length of shoot and root was measured in cms scale.

3.13.1.2 Leaf area per plant

Leaf area was ascertained by gravimetric method. The leaf area of few leaves from each treatment was determined by tracing on graph sheet and dry mass for these samples were recorded. The leaf area per plant was computed by using leaf dry mass per plant and the dry mass of those leaves for which the area was estimated using the following formula:

$$LA = \frac{LA_1 \times W_2}{W_1} cm^2$$

Where, LA_1 = Leaf area of the leaves traced on graph paper.

W_1 = Dry mass of the leaves for which area was traced on graph paper.

W_2 = Total leaf dry mass per plant

3.13.1.3 Fresh and dry mass of root and shoot per plant

Fresh mass of the root and shoot was determined with the help of electrical balance whereas, for dry mass the samples were kept in an oven run at 80°C for 48 h. After 48 h, the samples were weighed on electrical balance to ascertain their dry mass.

3.13.2 Biochemical Parameters

3.13.2.1 SPAD chlorophyll

SPAD chlorophyll meter (Minolta) was used to assess the SPAD value for chlorophyll in the leaves.

3.13.2.2 Photosynthetic measurements

The photosynthetic parameters (photosynthetic rate, stomatal conductance, water use efficiency, internal CO_2 concentration, and transpiration rate) were measured by using portable photosynthesis system (LI-COR 6400, Lincoln, NE, USA). The measurements were made on uppermost fully expanded leaves of the main branch in the sampled plants between 11 to 12 hours under clear sun light.

3.13.2.3 Nitrate reductase (NR) activity

The activity of NR was measured following the method laid down by Jaworski (1971) in fresh leaf. The leaves were cut into small pieces (1 cm^2). These samples were weighed (200mg) and transferred to plastic vials. To each vial 2.5 ml of phosphate buffer (Appendix 1.1) and 0.5 ml of potassium nitrate solution (Appendix 1.2) was added followed by the addition of 2.5 ml of 5% isopropanol (Appendix 1.3). These vials were incubated in BOD incubator for 2 hours at 30±2°C in dark. Incubated mixture (0.4ml) was taken in a test tube to which 0.3 ml each of sulphanilamide solution (Appendix 1.4) and NED-HCl (Appendix 1.5) were added.

The test tube was left for 20 minutes, for maximum colour development. The mixture was diluted to 5 ml with DDW. The absorbance was read at 540 nm on spectrophotometer (Milton & Roy, USA). A blank was run simultaneously with each sample. Standard curve was plotted by using known graded concentration of sodium nitrite solution. The absorbance of each sample was compared with that of calibration curve and NR activity [n mol NO_2 $g^{-1}h^{-1}$] on fresh mass basis was recorded.

3.13.2.4 Carbonic anhydrase (CA) activity

The activity of CA in the leaves was measured following the method described by Dwivedi and Randhawa (1974). The fresh leaves were cut into small pieces and 200 mg of these pieces was weighed and transferred to petriplates. The leaf pieces were cut further into smaller pieces in 10 ml of 0.2m cystein hydrochloride (Appendix 2.1) and left at 4°C for 20 minutes. The leaf pieces were blotted and transferred to a test tube containing 4 ml of phosphate buffer of pH 6.8 (Appendix 2.2). To this test tube 4 ml of 0.2M sodium bicarbonate (Appendix 2.3) solution and 0.2 ml of 0.002% bromothymol blue (Appendix 2.4) were added. The test tube was shaken gently and left at 4°C for 20 minutes. Carbon dioxide liberated by the catalytic action of CA on $NaHCO_3$ was estimated by titrating the reaction mixture against 0.05N HCl (Appendix 2.5) using methyl red (Appendix 2.6) as an indicator. In each sample the quantity of HCl used to neutralize reaction mixture was noted and difference was calculated. A blank consisting of all the above components of reaction mixture, except the leaf sample, was run simultaneously with each set of samples. The activity of the enzyme was calculated by putting the values in the formula:

$$\frac{V \times 22 \times N}{W} \text{ [mol } (CO_2) \text{ kg}^{-1} \text{ (Leaf fresh mass)s}^{-1}\text{]}$$

Where, V = difference in volume (ml of HCl used in control and test sample titration).

22 = equivalent mass of CO_2

N = Normality of HCl

W = Fresh mass of tissue used

3.13.2.5 Estimation of antioxidative enzymes

3.13.2.5.1 Extraction

Leaf tissue (500mg) was homogenized in 5 ml of 50 mM phosphate buffer (pH 7.0) containing 1% polyvinyl pyrrolidone. The homogenate was centrifuged at 15,000 rpm for 10 minutes at 5°C and the supernatant was used for the estimation of peroxidase, catalase and superoxide dismutase activities.

3.13.2.5.2 Estimation of catalase (CAT)

The estimation of CAT was done by permanganate titration method (Chance and Maehly, 1956). Five ml of phosphate buffer (Appendix 3.1), 1 ml of 0.1M H_2O_2 (Appendix 3.2) and 1 ml of enzyme extract were mixed and incubated at 25°C for 1 minute. Then 10 ml of 2% H_2SO_4 (Appendix 3.3) was added. The mixture was titrated against 0.1N potassium permanganate (Appendix 3.4) to find the residual H_2O_2 until a purple colour persists for at least 15 seconds. Similarly, a control set was stopped by the addition of H_2SO_4, prior to the addition of enzyme extract.

3.13.2.5.3 Estimation of peroxidase (POX)

The activity of POX was measured following the method laid down by Chance and Maehley (1956) in fresh leaf samples. Three ml of pyrogallol phosphate buffer (Appendix 4.1), 0.1 ml of enzyme extract and 0.5 ml of 1% H_2O_2 were mixed in a cuvette and a change in absorbance, at 20 seconds interval for a period of 3

minutes was read at 420 nm on a spectrophotometer. The control set was prepared by boiling the enzyme extract.

3.13.2.5.4 Estimation of superoxide dismutase (SOD)

The activity of SOD was measured by the method of Beauchamp and Fridovich (1971). A 3 ml of reaction mixture containing 1 ml of 50 mM phosphate buffer (Appendix 5.1), 0.5 ml of 13 mM methionine (Appendix 5.2), 0.5 ml of 75 mM NBT (Appendix 5.3), 0.5 ml of 2 µM riboflavin (Appendix 5.4), 0.5 ml of 0.1 mM EDTA (Appendix 5.5) and 0.1 ml of enzyme extract was made. Riboflavin was added in the last. The absorbance of the reaction mixture was read at 560 nm on a spectrophotometer.

3.13.2.6 Proline content

The proline content in fresh leaves was estimated following the procedure used by Bates *et al.* (1973). Fresh sample (0 5 g) was homogenized in a mortar with 5 ml of 3% sulphosalicylic acid (Appendix 6.1). The homogenate was filtered through Whatman filter paper No. 2 and collected in a test tube with two washings. Five ml of sulphosalicyclic acid, 2 ml each of glacial acetic acid and acid ninhydrin (Appendix 6.2) was added to 2 ml of the above extract. This mixture was heated in boiling water bath for 1 hour. The reaction was terminated by transferring the test tubes to ice box. Four ml of toluene was mixed to the reaction mixture with vigorously shaking for 20-30 seconds. The chromophore (toluene) layer was aspirated and warmed to room temperature. The absorbance of red colour was read at 520 nm against a reagent blank. The amount of proline in the sample was calculated by using a standard curve prepared from pure proline (range 0.1-36 µ mol) and expressed on fresh mass basis of the sample.

$$\mu \text{ moles of proline } g^{-1} \text{ tissue} = \frac{\mu g \text{ proline } ml^{-1} \times ml^{-1} \text{ toluene}}{115.5} \times \frac{5}{g \text{ (sample)}}$$

Where 115.5 is the molecular mass of the proline

3.14 Statistical analysis

The experiment was conducted according to simple randomized design. Each treatment was represented by ten pots. Three observations from three different pots were recorded per treatment. The treatment means were compared by analysis of variance using SPSS software version 10 (SPSS, Chicago, IL, USA). Least significant difference (LSD) was calculated at 5% level of probability.

Contents

CHAPTER-4

RESULTS

4.1 Experiment 1

4.1.1 Root and shoot length

Plants raised from the seeds given pre-sowing seed soaking treatment in NaCl showed significant reduction in length of root and shoot. The degree of damage caused by NaCl was more pronounced at the early stage of the growth i.e. 45 days after sowing (DAS) than at later stage of growth (60 DAS). The highest concentration of salt i.e. 150 mM generated maximum toxicity and decreased length of root by 45.2% and that of shoot 44.6% at 45 DAS (Tables 1-2).

4.1.2 Fresh and dry mass of root

The fresh and dry mass of root also followed a similar pattern as was observed in the case length of root and shoot, which decreased significantly with increasing concentration of salt. Plants that were raised from the seeds soaked in 150 mM of NaCl showed maximum damage and decreased the values of fresh mass by 44.63% and 33.16% and dry mass by 72.13% and 50.5% at 45 DAS and 60 DAS respectively over their control (Table 1).

4.1.3 Fresh and dry mass of shoot

With the advancement of age the mass of the plant increased. The degree of damage caused by the salinity treatment was more pronounced at the early stage of growth than at latter stage. A decrease in fresh mass of shoot by 11.1, 23.5% and 33.9% and that of dry mass 18.48, 32.91 and 50.0% at 45 DAS in response to 50, 100 or 150 mM NaCl was recorded. Similar trend of response was also followed at 60 DAS (Table 2).

Table 1 **Effect of pre-sowing seed soaking treatment of NaCl (0, 50, 100 or 150 mM) for 8 h on the length (cm), fresh and dry mass (g) of root of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 days after sowing (DAS)**

	Root length		Root fresh mass		Root dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	6.00	9.11	1.21	1.87	0.244	0.400
NaCl (50 mM)	5.40	8.29	0.86	1.56	0.155	0.299
NaCl (100 mM)	4.61	7.50	0.74	1.42	0.110	0.240
NaCl (150 mM)	3.29	7.00	0.67	1.25	0.068	0.198
LSD at 5%	0.54	1.00	0.086	0.19	0.016	0.038

Table 2 **Effect of pre-sowing seed soaking treatment of NaCl (0, 50, 100 or 150 mM) for 8 h on the length (cm), fresh and dry mass (g) of shoot of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Shoot length		Shoot fresh mass		Shoot dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	9.02	12.42	3.15	5.80	0.790	1.392
NaCl (50 mM)	7.80	11.00	2.80	5.50	0.644	1.210
NaCl (100 mM)	6.42	9.50	2.41	5.15	0.530	1.030
NaCl (150 mM)	5.00	8.11	2.08	4.30	0.395	0.788
LSD at 5%	0.64	1.36	0.35	0.76	0.12	0.15

4.1.4 Leaf area

Leaf area exhibited an increasing trend with the advancement of age from 45 to 60 DAS. However, a linear decrease in the leaf area was observed with the increasing concentration of salinity (Table 3). The plants raised from the seeds given pre sowing seed soaking treatment in 150 mM of NaCl showed 44.2% decrease in leaf area at 45 day stage. Similar trend was followed at 60 DAS also.

4.1.5 Relative water content (RWC)

As evident from table 3 that the values for RWC decreased with the increasing concentration of salinity. The lowest concentration of salt i.e. 50 mM was found to be the least toxic and decreased the values for RWC by 9.54% and 7.67% at 45 and 60 DAS over their respective controls. However, the highest concentration of salt showed 27.52 and 13.76% decreased at two growth stages respectively over their control plants.

4.1.6 SPAD chlorophyll

Pre-sowing seed soaking in differential concentration of NaCl caused significant reduction in the SPAD chlorophyll values (Table 3). The highest concentration of salt i.e. 150 mM generate maximum toxicity at both the stages of growth and decreased the values of SPAD chlorophyll by 32.80% and 28.3% at 45 and 60 DAS over their respective control.

4.1.7 Photosynthetic rate (P_N)

The leaves of the plants that were raised from the seeds given pre-sowing seed soaking in different concentration of NaCl showed a linear decrease in P_N with the increasing concentration of salt (Table 4). The degree of damage caused by the salt was maximum in 150 mM of NaCl where the values were decreased by 32.93% and 18.36% at 45 and 60 DAS respectively over the control.

Table 3 **Effect of pre-sowing seed soaking treatment of NaCl (0, 50, 100 or 150 mM) for 8 h on the leaf area (cm^2), relative water content (RWC; %) and SPAD Chlorophyll of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Leaf area		RWC		SPAD Chlorophyll	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	5.70	7.00	73.4	82.1	38.1	45.8
NaCl (50 mM)	4.36	5.68	66.4	75.8	33.6	41.6
NaCl (100 mM)	3.90	5.20	62.1	72.3	30.6	38.3
NaCl (150 mM)	3.18	4.50	53.2	63.3	25.6	32.8
LSD at 5%	0.47	0.57	6.52	7.30	2.89	4.52

Table 4 **Effect of pre-sowing seed soaking treatment of NaCl (0, 50, 100 or 150 mM) for 8 h on the photosynthetic rate [P_N; μ mol (CO_2) $m^{-2}s^{-1}$], stomatal conductance (g_s; mol $m^{-2}s^{-1}$) and internal CO_2 concentration (C_i; ppm) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	P_N		g_s		C_i	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	7.47	9.80	0.060	0.080	324	347
NaCl (50 mM)	6.49	9.06	0.050	0.070	267	308
NaCl (100 mM)	5.55	8.46	0.043	0.063	248	281
NaCl (150 mM)	5.01	8.00	0.037	0.056	224	258
LSD at 5%	0.67	0.75	0.003	0.005	21.7	23.8

4.1.8 Stomatal conductance (g_s)

The data presented in table 4 showed that plant raised from the seeds given pre-sowing seed soaking in different concentration of NaCl significantly decreased the g_s, more effectively at early stage of growth than at latter stage. Among the concentrations, the highest one i.e. 150 mM was found to be most toxic and decreased the values of g_s by 38.3% at 45 day stage. However the degree of damage caused by salinity decreased with the advancement of age and only 30.0% (150 mM NaCl) was observed at 60 DAS over their control plants.

4.1.9 Internal CO_2 concentration (C_i)

This parameter also follows a similar trend of response as that of g_s (Table 4). Here again, the degree of damage caused by the salinity decreased with the increasing age of the plant and only 11.2%, 19.0% and 25.6% decreased was observed at 60 DAS over their control plant in response to 50, 100 and 150 mM NaCl and at 45 DAS damage was comparatively higher.

4.1.10 Water use efficiency (WUE)

It is evident from table 5 that plants given pre-sowing seed soaking in different concentration of NaCl showed a significant reduction in WUE. However, the degree of damage caused by NaCl was more pronounced at early stage of growth than at latter stage. Out of three tested concentrations of NaCl (50, 100 or 150 mM) the highest concentration was found to be the most toxic

4.1.11 Transpiration rate (E)

The data presented in table 5 showed that pre-sowing seed soaking treatment caused a significant decreased in E of the resulting plants. The degree of damage caused by NaCl treatment increased with the increasing concentration and thus

Table 5 **Effect of pre-sowing seed soaking treatment of NaCl (0, 50, 100 or 150 mM) for 8 h on the water use efficiency (WUE) transpiration rate (E; $mmolm^{-2}sec^{-1}$) and nitrate reductase (NR) activity [n mol NO_2 $g^{-1}h^{-1}$ leaf F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	WUE		E		NR activity	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	0.261	0.297	1.96	2.72	329	376
NaCl (50 mM)	0.234	0.277	1.77	2.55	288	342
NaCl (100 mM)	0.202	0.248	1.61	2.37	267	320
NaCl (150 mM)	0.170	0.230	1.43	2.16	213	262
LSD at 5%	0.018	0.017	0.15	0.19	23.0	28.4

Table 6 **Effect of pre-sowing seed soaking treatment of NaCl (0, 50, 100 or 150 mM) for 8 h on the carbonic anhydrase (CA) activity [mol (CO_2) $kg^{-1}s^{-1}$], peroxidase (POX) activity [g^{-1}(F.M.)] and catalase (CAT) activity [μ mol H_2O_2 decomposed g^{-1} (F.M.)] in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	CA activity		POX		CAT	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	3.41	3.92	9.76	11.42	379	428
NaCl (50 mM)	3.00	3.59	10.8	12.34	413	453
NaCl (100 mM)	2.54	3.15	11.9	13.32	445	483
NaCl (150 mM)	2.02	2.72	13.8	15.28	493	533
LSD at 5%	0.20	0.25	0.97	1.30	38.67	39.36

maximum damage was observed in 150 mM concentration at both the growth stage. However, the degree of damage decreased with the advancement of plant age.

4.1.12 Nitrate reductase (NR) activity

Plants grown from the seeds given pre-sowing seed soaking in different concentration of NaCl (50, 100 or 150 mM) showed significantly lower NR activity over their control plant (Table 5). However, the degree of damage caused by the lower concentration of NaCl was found to be least toxic and decreased the activity of NR only by 12.46 and 9.04% at 45 and 60 DAS respectively over their control plants.

4.1.13 Carbonic anhydrase (CA) activity

The activity of CA increased with the advancement of plant age (45 to 60 DAS) but showed significant decreased with the increasing concentration of NaCl (Table 6). Out of the different concentration of salt the highest concentration (150 mM) was found to be most toxic and decreased the activity of CA by 40.7% and 30.6% at 45 and 60 DAS over their control plants.

4.1.14 Peroxidase (POX) activity

The activity of POX showed completely different trend as compared to all the other parameters studied earlier (Table 6). The activity of POX increased with the increased concentration of NaCl. Plants that were raised from the seeds given pre-sowing seed soaking in 150 mM of NaCl possessed highest POX activity at both the stages of growth and it showed 41.4% and 33.8% increase over their control plants at 45 and 60 DAS respectively.

4.1.15 Catalase (CAT) activity

Data depicted in table 6 indicate that the activity of CAT increased in response to both, age of the plant as well as with the increasing concentration of NaCl. Thus the

plants that were fed with 150 mM of NaCl possessed highest values for CAT activity. Control plant possessed lowest values for CAT activity.

4.1.16 Superoxide dismutase (SOD) activity

The activity of SOD also followed a similar trend of response as that of POX and CAT (Table 7). Leaves of the plants that were raised from the seeds given pre-sowing seed soaking in 150 mM of NaCl possessed maximum SOD activity and was 34.2% and 24.1% higher over their respective control at 45 and 60 DAS.

4.1.17 Proline content

It is evident from table 7 that the proline content of the leaves was comparatively higher in the plants that were raised from the seeds given pre-sowing seed soaking in differential concentration of NaCl. The values increased with the increased concentration of salt as well as age of the plants. Thus maximum values for proline content were recorded from the plants at 60 DAS that were fed with 150 mM of NaCl.

4.2 Experiment 2

4.2.1 Root and shoot length

The data in table 8 and 9 indicate that soil applied NaCl resulted in a significant decrease in the length of the root and shoot of resulting plants. The highest concentration of NaCl (8.7 mg Kg^{-1}) had the maximum reduction in the length of root and shoot at 45 days after sowing (DAS). The per cent reduction in the root and shoot length was higher at 45 DAS (37.0 and 30.7%) than at 60 DAS (20.4 and 27.0%) over their control.

4.2.2 Fresh and dry mass of root

The plant raised from soil received different concentrations of NaCl exhibited an inhibition in root fresh and dry mass. The plant showed different response to

Table 7 **Effect of pre-sowing seed soaking treatment of NaCl (0, 50, 100 or 150 mM) for 8 h on the superoxide dismutase (SOD) activity (units g^{-1} FM) and proline content (mg g^{-1} F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	SOD		Proline content	
	45 DAS	60 DAS	45 DAS	60 DAS
Control	108	120	11.4	12.5
NaCl (50 mM)	119	128	13.1	13.9
NaCl (100 mM)	132	140	14.9	15.6
NaCl (150 mM)	145	149	16.8	17.3
LSD at 5%	12.73	13.79	1.37	1.48

different concentrations of NaCl. The lowest concentration (2.9 mg Kg^{-1}) showed the least inhibition and was 41.2 and 64.6% and 24.7 and 48.20% in root fresh mass and dry mass over their controls at 45 and 60 DAS respectively (Table 8).

4.2.3 Fresh and dry mass of shoot

The data presented in table 9 shows that the application of different concentrations of NaCl (2.9, 5.8 or 8.7 mg Kg^{-1}) to soil significantly reduced the shoot fresh and dry mass. The reduction was proportional to the increasing concentration of the salt in the soil. The lowest concentration showed least decrease and maximum decrease was reported at highest concentration.

4.2.4 Leaf area

The data depicted in table 10 shows that soil applied NaCl treatment caused a significant decrease in the leaf area of the resulting plants. As the concentration of NaCl increased the degree of inhibition also increased.

4.2.6 Relative water content (RWC)

It is evident from table 10 that the plants raised from the soil mixed with different concentrations of NaCl showed a significant reduction in RWC of the leaves. The reduction in RWC was maximum at highest concentration of NaCl.

4.2.6 SPAD chlorophyll

The plants grown from soil amended with different concentrations of NaCl possessed significantly lower level of SPAD chlorophyll than the unstressed control (Table 10). Out of the used NaCl concentrations (2.9, 5.8 or 8.7 mg Kg^{-1}), the 8.7 mg Kg^{-1} was the most toxic. This concentration decreased the level of SPAD chlorophyll by 23.6% at 45 DAS and 18.6% at 60 DAS over their control plants.

Table 8 **Effect of soil applied NaCl (0, 2.9, 5.8 or 8.7 mg Kg^{-1}) on the length (cm), fresh and dry mass (g) of root of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Root length		Root fresh mass		Root dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	6.24	9.26	1.09	1.70	0.280	0.390
NaCl (2.9 mg Kg^{-1})	5.71	8.66	0.82	1.50	0.201	0.331
NaCl (5.8 mg Kg^{-1})	5.05	7.86	0.72	1.36	0.162	0.261
NaCl (8.7 mg Kg^{-1})	3.93	7.37	0.64	1.28	0.099	0.202
LSD at 5%	0.78	1.20	0.11	0.18	0.02	0.03

Table 9 **Effect of soil applied NaCl (0, 2.9, 5.8 or 8.7 mg Kg^{-1}) on the length (cm), fresh and dry mass (g) of shoot of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Shoot length		Shoot fresh mass		Shoot dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	9.26	12.59	3.20	5.60	0.798	1.342
NaCl (2.9 mg Kg^{-1})	8.10	11.42	2.90	5.37	0.672	1.208
NaCl (5.8 mg Kg^{-1})	7.00	10.12	2.69	5.14	0.571	1.053
NaCl (8.7 mg Kg^{-1})	6.41	9.19	2.28	4.48	0.468	0.892
LSD at 5%	0.89	1.54	0.40	0.77	0.089	0.162

Table 10 **Effect of soil applied NaCl (0, 2.9, 5.8 or 8.7 mg Kg^{-1}) on the leaf area (cm^2), RWC (%) and SPAD chlorophyll of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Leaf area		RWC		SPAD chlorophyll	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	5.26	6.84	75.6	83.4	39.4	44.6
NaCl (2.9 mg Kg^{-1})	4.50	6.14	69.8	78.5	36.1	41.8
NaCl (5.8 mg Kg^{-1})	3.80	5.62	66.0	75.7	34.4	40.4
NaCl (8.7 mg Kg^{-1})	3.17	4.79	59.3	68.7	30.1	36.3
LSD at 5%	0.57	0.56	5.40	6.24	2.98	3.55

Table 11 **Effect of soil applied NaCl (0, 2.9, 5.8 or 8.7 mg Kg^{-1}) on the P_N [μ mol (CO_2) $m^{-2}s^{-1}$], g_s (mol $m^{-2}s^{-1}$) and C_i (ppm) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	P_N		g_s		C_i	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	7.56	9.71	0.061	0.081	325	345
NaCl (2.9 mg Kg^{-1})	6.82	9.20	0.053	0.075	281	312
NaCl (5.8 mg Kg^{-1})	6.33	8.82	0.046	0.065	264	294
NaCl (8.7 mg Kg^{-1})	5.58	8.25	0.041	0.061	241	274
LSD at 5%	0.56	0.79	0.003	0.004	22.9	25.8

4.2.7 Photosynthetic rate (P_N)

The leaves of the plants that were raised from the seeds sown in soil amended with 8.7 mg Kg^{-1} of NaCl, photosynthesized at the minimum rate as compared to the control. Photosynthetic rate of the plants decreased with the increasing concentration of the NaCl. Even at lowest concentration it was 9.7 and 5.2% lower than control at 45 and 60 DAS (Table 11).

4.2.8 Stomatal conductance (g_s)

Stomatal conductance of the leaf decreased with the increasing concentration of the NaCl in the soil (Table 11). The lowest concentration (2.9 mg Kg^{-1}) of NaCl caused least damage and reduced the g_s by 13.1% and 7.4% when compared to the control at 45 DAS and 60 DAS.

4.2.9 Internal CO_2 concentration (C_i)

The internal CO_2 concentration (Table 11) followed a pattern similar to that of g_s. The decrease in the C_i is proportionate to the concentration of NaCl. The per cent reduction in the values of C_i for 2.9, 5.8 or 8.7 mg Kg^{-1} were 13.5, 18.7 and 25.8% respectively, when compared to their respective control at 45 DAS.

4.2.10 Water use efficiency (WUE)

The WUE gradually decreased as the concentration of NaCl increased (Table 12). The lowest reduction was observed at a salt concentration of 2.9 mg Kg^{-1} which was 8.6 and 4.8% at 45 and 60 DAS over their respective control, whereas, highest reduction was recorded at a salt concentration of 8.7 mg Kg^{-1} of soil.

4.2.11 Transpiration rate (E)

Transpiration rate also shows a similar pattern as that of WUE. Maximum reduction was noted at highest concentration of salt and minimum at lower concentration (Table 12).

4.2.12 Nitrate reductase (NR) activity

The activity of leaf NR was significantly decreased in all the NaCl concentrations. Out of the concentrations used, the 2.9 mg Kg^{-1} was least toxic and decreased the NR activity by 8.53% and 5.86% at 45 and 60 DAS respectively, below the control. The highest concentration of NaCl showed maximum reduction in NR activity (Table 12).

4.2.13 Carbonic anhydrase (CA) activity

A significant decrease in the activity of CA was recorded in response to the soil applied NaCl treatment. The highest concentration of NaCl (8.7 mg Kg^{-1}) decreased the CA activity by 32.53% and 25.96% at 45 and 60 DAS over their respective control.

4.2.14 Peroxidase (POX) activity

The activity of POX exhibited a trend which was converse to that of all other parameters explained earlier. The POX activity was positively affected by NaCl treatment (Table 13). The plants raised from soil applied with different NaCl concentrations (2.9, 5.8 or 8.7 mg Kg^{-1}) possessed higher activities of the enzymes which were 7.48, 17.98 and 30.04 at 45 DAS and 5.48, 11.68 and 24.07% at 60 DAS respectively over their respective controls.

4.2.15 Catalase (CAT) activity

The activity of enzyme CAT (Table 13) exhibited a similar response as that of POX. Leaves of the plants that were raised from the seeds sown in soil mixed with NaCl (2.9, 5.8 or 8.7 mg Kg^{-1}) possessed 6.31, 11.31 and 21.32% higher CAT activity over their respective controls at 45 DAS. Almost similar increase was recorded at 60 DAS.

Table 12 **Effect of soil applied NaCl (0, 2.9, 5.8 or 8.7 mg Kg^{-1}) on the WUE, E ($mmolm^{-2}sec^{-1}$) and NR activity [n mol NO_2 $g^{-1}h^{-1}$ leaf F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	WUE		E		NR activity	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	0.257	0.293	1.96	2.71	328	375
NaCl (2.9 mg Kg^{-1})	0.235	0.279	1.81	2.60	300	353
NaCl (5.8 mg Kg^{-1})	0.216	0.262	1.68	2.43	279	330
NaCl (8.7 mg Kg^{-1})	0.188	0.238	1.54	2.25	234	293
LSD at 5%	0.020	0.025	0.14	0.21	24.4	28.9

Table 13 **Effect of soil applied NaCl (0, 2.9, 5.8 or 8.7 mg Kg^{-1}) on the CA activity [mol (CO_2) $kg^{-1}s^{-1}$], POX activity [g^{-1}(F.M.)] and CAT activity [μ mol H_2O_2 decomposed g^{-1} (F.M.)] in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	CA activity		POX		CAT	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	3.35	3.89	9.62	11.30	380	425
NaCl (2.9 mg Kg^{-1})	3.00	3.65	10.34	11.92	404	441
NaCl (5.8 mg Kg^{-1})	2.73	3.35	11.35	12.64	423	465
NaCl (8.7 mg Kg^{-1})	2.26	2.88	12.51	14.02	461	500
LSD at 5%	0.24	0.30	0.95	1.10	36.1	38.2

4.2.16 Superoxide dismutase (SOD) activity

The observations depicted in table 14 clearly indicated that the NaCl treatment resulted in a significant increase in the activity of SOD. The lowest concentration of NaCl (2.9 mg Kg^{-1}) showed least increase; where as maximum increase was recorded at highest concentration (8.7 mg Kg^{-1}).

4.2.17 Proline content

The leaves of the plants raised from the seeds sown in soil amended with NaCl concentrations (2.9, 5.8 or 8.7 mg Kg^{-1}) possessed significantly higher proline content (Table 14). The highest concentration of NaCl (8.7 mg Kg^{-1}) generated maximum proline content both after 45 and 60 DAS.

Experiment 3

4.3.1 Root and shoot length

The length of the root and shoot increased with the advancement of the age of the plants and was significantly increased by the pre-sowing seed soaking in sodium nitroprusside (SNP). Among the three different concentrations of SNP the 10^{-5}M proved to be the best and showed a maximum increase in the values of root and shoot length both at 45 and 60 DAS.

4.3.2 Fresh and dry mass of root

The pre-sowing seed soaking in different concentrations of SNP (10^{-4}, 10^{-5} or 10^{-6} M) significantly increased the root fresh and dry mass (Table 15). Out of the tested concentration 10^{-5} M of SNP proved to be the best and increased the root fresh and dry mass by 34.4 and 39.2% at 45 DAS and by 26.6 and 33.1% at 60 DAS over their respective controls.

Table 14 **Effect of soil applied NaCl (0, 2.9, 5.8 or 8.7 mg Kg^{-1}) on the SOD activity (units g^{-1} FM) and proline content (mg g^{-1} F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	SOD		Proline content	
	45 DAS	60 DAS	45 DAS	60 DAS
Control	110	119	11.10	12.60
NaCl (2.9 mg Kg^{-1})	119	124	12.26	13.55
NaCl (5.8 mg Kg^{-1})	129	132	13.48	15.00
NaCl (8.7 mg Kg^{-1})	141	145	15.03	16.37
LSD at 5%	10.1	11.1	1.18	1.30

4.3.3 Fresh and dry mass of shoot

Fresh and dry mass of shoot exhibited an increase as the growth progressed (Table 16). Pre-sowing seed treatment with SNP showed a significant increase in the shoot fresh and dry mass. The maximum increase was recorded in the plants raised from the seeds soaked in 10^{-5} M of SNP and was about 40.4% (shoot fresh) and 26.7% (dry mass) at 45 DAS.

4.3.4 Leaf area

It is evident from table 17 that as the growth progressed from 45 to 60 DAS, the leaf area of the plant was increased irrespective of the treatment. However, the plants raised from the seeds pre-treated with 10^{-5} M of SNP possessed maximum leaf area at both (45 and 60 DAS) the sampling stages.

4.3.5 Relative water content (RWC)

The data presented in table 17 express that the plants raised from the seeds given pre-sowing seed treatment with SNP, showed significant increase in RWC. The maximum increase was recorded in the plants raised from the seeds soaked in 10^{-5} M of SNP at 45 and 60 DAS, which were 28.1% and 37.5% higher over their respective controls.

4.3.6 SPAD chlorophyll

With the advancement of the age of the plant from 45 to 60 days, SPAD chlorophyll increased (Table 17). Leaves of the plants raised from the seeds soaked in different concentration (10^{-4}, 10^{-5} or 10^{-6} M) of SNP possesses significantly higher SPAD value over control and maximum increase was recorded in the leaves of the plants raised from the seeds soaked in 10^{-5} M of SNP at 45 and 60 DAS.

Table 15 **Effect of pre-sowing seed soaking treatment in sodium nitroprusside (SNP) (10^{-4}, 10^{-5} or 10^{-6} M) for 8 h on the length (cm), fresh and dry mass (g) of root of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Root length		Root fresh mass		Root dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	6.24	9.15	1.25	1.84	0.227	0.399
SNP (10^{-4} M)	7.71	10.91	1.57	2.25	0.297	0.490
SNP (10^{-5} M)	8.14	11.54	1.68	2.33	0.316	0.531
SNP (10^{-6} M)	7.32	10.17	1.49	2.15	0.277	0.468
LSD at 5%	1.27	1.81	0.24	0.33	0.048	0.079

Table 16 **Effect of pre-sowing seed soaking treatment in SNP (10^{-4}, 10^{-5} or 10^{-6} M) for 8 h on the length (cm), fresh mass and dry mass (g) of shoot of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Shoot length		Shoot fresh mass		Shoot dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	9.32	12.56	3.07	5.21	0.789	1.364
SNP (10^{-4} M)	12.36	16.03	4.07	6.61	1.080	1.768
SNP (10^{-5} M)	13.19	17.01	4.31	7.01	1.141	1.902
SNP (10^{-6} M)	11.62	15.27	3.79	6.16	1.000	1.656
LSD at 5%	1.97	2.55	0.65	1.06	0.16	0.27

4.3.7 Photosynthetic rate (P_N)

The leaves of plants raised from the pre-sowing seed soaking treatment of SNP (10^{-5} M) photosynthesized at the maximum rate (Table 18). A progressive increased in the values was observed with the advancement of plant age. SNP showed a concentration dependent variation in photosynthetic value in the order of 10^{-5} M > 10^{-4} M > 10^{-6} M with respect to the control.

4.3.8 Stomatal conductance (g_s)

Stomatal conductance of the leaf increased in the plants raised from the pre-sowing soaking treatment with different concentration of SNP. This increase was maximum at 10^{-5} M of SNP, whereas, other two concentrations were statistically at par.

4.3.9 Internal CO_2 concentration (C_i)

Pre-sowing seed treatment with SNP increased C_i in the resulting plants in a concentration dependent manner (Table 18). An increase in the C_i was observed at both the stages of growth (45 and 60 DAS). SNP (10^{-5}M) enhanced the values of C_i 16.1% and 13.5% at 45 and 60 DAS respectively over their controls.

4.3.10 Water use efficiency (WUE)

The value of WUE increased as the age of the plants advanced from day 45 to 60 (Table 19). Out of three concentrations of SNP, 10^{-5} M generated a better response and enhanced the values of WUE by 49.2 and 34.5% at 45 and 60 DAS over their control plants respectively.

4.3.11 Transpiration rate (E)

The E of the plants expressed a response similar to that of WUE, during the growth period (Table 19). The E of the plants resulting from SNP treated seeds was significantly greater than water treated controls. A significant increase in E was

Table 17 **Effect of pre-sowing seed soaking treatment in SNP (10^{-4}, 10^{-5} or 10^{-6} M) for 8 h on the leaf area (cm^2), RWC (%) and SPAD chlorophyll of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Leaf area		RWC		SPAD chlorophyll	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	5.40	6.81	75.1	84.5	37.9	45.0
SNP (10^{-4} M)	6.50	7.51	91.7	112.4	48.6	56.0
SNP (10^{-5} M)	6.75	7.92	96.2	116.2	52.1	56.7
SNP (10^{-6} M)	6.00	7.21	87.9	107.6	46.0	53.2
LSD at 5%	0.74	0.79	7.52	9.20	4.28	4.95

Table 18 **Effect of pre-sowing seed soaking treatment in SNP (10^{-4}, 10^{-5} or 10^{-6} M) for 8 h on the P_N [μ mol (CO_2) $m^{-2}s^{-1}$], g_s (mol $m^{-2}s^{-1}$) and C_i (ppm) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	P_N		g_s		C_i	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	7.01	9.12	0.062	0.081	324	348
SNP (10^{-4} M)	7.96	10.01	0.082	0.101	361	381
SNP (10^{-5} M)	8.58	10.69	0.086	0.108	376	395
SNP (10^{-6} M)	7.68	9.76	0.075	0.094	351	368
LSD at 5%	0.74	0.96	0.006	0.008	30.1	32.5

recorded in the plants supplemented with SNP (10^{-4}, 10^{-5} or 10^{-6} M) concentrations and maximum values were recorded in the plants raised from the seeds soaked in 10^{-5} M of SNP.

4.3.12 Nitrate reductase (NR) activity

It is evident from table 19 that the plants raised from the seeds given pre-sowing seed soaking treatment with SNP possessed significantly higher NR activity at both the stages of growth (45 and 60 DAS) as compared to their respective controls. A maximum increase in the NR activity was recorded in the leaves of the plant raised from the seeds soaked in 10^{-5} M of SNP at both the sampling stages.

4.3.13 Carbonic anhydrase (CA) activity

The activity of CA increased with the age of the plants (Table 20). The SNP (10^{-4}, 10^{-5} or 10^{-6} M) increased the value of CA by 30.0, 39.4 and 24.7% at 45 DAS. The 10^{-5}M of SNP was proved to be the best at both the stages of plant growth (45 and 60 DAS).

4.3.14 Peroxidase (POX) activity

The data depicted in the table 20 clearly revealed an increase in the activity of POX, as growth progressed from 45 to 60 days and also in response to SNP (10^{-4}, 10^{-5} or 10^{-6} M). Out of three concentrations (10^{-4}, 10^{-5} or 10^{-6} M) of SNP, 10^{-5} M proved best and enhanced the POX activity by 32.48 and 26.47 at 45 and 60 DAS.

4.3.15 Catalase (CAT) activity

The activity of enzyme CAT (Table 20) exhibited a similar response as that of POX. The leaves of the plants that were raised from the seeds given pre sowing seed soaking SNP treatment possessed higher CAT activity and it increased as the growth progressed (45 to 60 DAS). The 10^{-5}M of SNP showed maximum increase irrespective of the days of sampling.

Table 19 **Effect of pre-sowing seed soaking treatment in SNP (10^{-4}, 10^{-5} or 10^{-6} M) for 8 h on the WUE, E ($mmolm^{-2}sec^{-1}$) and NR activity [n mol NO_2 $g^{-1}h^{-1}$ leaf F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	WUE		E		NR activity	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	0.240	0.290	1.95	2.76	326	376
SNP (10^{-4} M)	0.330	0.371	2.59	3.30	419	467
SNP (10^{-5} M)	0.358	0.390	2.80	3.54	448	494
SNP (10^{-6} M)	0.303	0.349	2.45	3.11	402	441
LSD at 5%	0.024	0.028	0.215	0.273	33.0	36.1

Table 20 **Effect of pre-sowing seed soaking treatment in SNP (10^{-4}, 10^{-5} or 10^{-6} M) for 8 h on the CA activity [mol (CO_2) $kg^{-1}s^{-1}$], POX activity [g^{-1}(F.M.)] and CAT activity [μ mol H_2O_2 decomposed g^{-1} (F.M.)] in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	CA activity		POX		CAT	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	3.40	3.87	10.90	12.20	378	427
SNP (10^{-4} M)	4.42	4.52	13.78	14.58	424	462
SNP (10^{-5} M)	4.74	4.66	14.44	15.43	433	473
SNP (10^{-6} M)	4.24	4.42	13.22	14.07	416	453
LSD at 5%	0.39	0.41	1.07	1.14	40.6	44.3

4.3.16 Superoxide dismutase (SOD) activity

It is evident from table 21 that the leaves of the plants raised from the seeds given pre sowing seed soaking treatment of SNP, possessed higher SOD activity in the plants, over their respective controls. The activity of the enzyme increased further with the advancement of age. 10^{-5}M of SNP showed 19.4 and 12.5% increase in SOD activity at 45 and 60 DAS respectively, over their controls.

4.3.17 Proline content

Compared with the control, the proline content increased in the plants treated with SNP (10^{-4}, 10^{-5} or 10^{-6} M). The 10^{-5}M of SNP proved better than 10^{-6} and 10^{-5} M SNP. The SNP (10^{-5}M) showed 17.30 and 14.52% increase in proline content at 45 and 60 DAS respectively, over their controls.

Experiment 4

4.4.1 Root and shoot length

The length of root and shoot increased with the advancement of the age of the plants and was significantly affected by the treatment (Tables 22-23). The plants exposed to foliar application of HBL (10^{-8}M) and EBL (10^{-8}M) showed maximum increase in their root and shoot length over their respective control at two stages of growth. Foliar application of EBL proved to be more effective in comparison to HBL.

4.4.2 Fresh and dry mass of root

The data depicted in table 22 indicates that fresh and dry mass of root exhibited an increase as the growth progressed. Foliar application of different concentrations of brassinosteroid analogues (HBL/EBL) favoured the fresh and dry mass of root. The per cent increase in the fresh mass of root by HBL/EBL (10^{-6}, 10^{-8} or 10^{-10} M) was found to be 0.80, 0.80, -% and 1.61, 2.41, 1.61% at 45 DAS. EBL at a

Table 21 **Effect of pre-sowing seed soaking treatment in SNP (10^{-4}, 10^{-5} or 10^{-6} M) for 8 h on the SOD activity (units g^{-1} FM) and proline content (mg g^{-1} F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	SOD		Proline content	
	45 DAS	60 DAS	45 DAS	60 DAS
Control	108	120	10.4	12.4
SNP (10^{-4} M)	124	132	11.07	13.5
SNP (10^{-5} M)	129	135	12.2	14.2
SNP (10^{-6} M)	122	130	11.05	13.3
LSD at 5%	9.78	10.42	0.92	1.06

concentration of 10^{-8}M was proved to be the best when compared with others at both the stages of growth.

4.4.3 Fresh and dry mass of shoot

Fresh and dry mass of shoot (Table 23) followed a pattern similar to that of the roots. Foliage of plants that received HBL/EBL (10^{-6}, 10^{-8} or 10^{-10}M) spray at 44 day stage possessed significantly improved shoot fresh and dry mass at 60 day stage. HBL (10^{-8}M) increased the fresh and dry mass of shoot by 50.5 % and 56.4% at 60 DAS compared to their respective control. EBL was found to be more effective than HBL.

4.4.4 Leaf area

Leaf area increased during the observation made from day 45 to 60 DAS (Table 24). The foliar application of three different concentrations of brassinosteroid analogues (HBL/EBL) increased the leaf area. The leaf area was increased 32.8% by HBL (10^{-8} M) and 46.5% by EBL (10^{-8} M) at 60 DAS over their respective controls.

4.4.5 Relative water content (RWC)

It is evident from table 24 that RWC exhibited an increase in its value with the foliar application of either of the brassinosteroid analogues (HBL/EBL). EBL was found to be more effective than HBL.

4.4.6 SPAD chlorophyll

Compared with 45 day stage, the leaf SPAD chlorophyll was more at 60 DAS (Table 24). Application of HBL (10^{-6}, 10^{-8} or 10^{-10}M) increased the SPAD by 40.2, 45.1 and 33.2% at 45 DAS over their respective control. EBL (10^{-8}M) increased the value of SPAD chlorophyll by 56.9% at 45 and 37.4 % at 60 DAS over the control value. Out of the two hormone analogues EBL was more effective than HBL.

Table 22 **Effect of foliar applied 28-homobrassinolide (HBL) or 24-epibrassinolide (EBL) (0, 10^{-6}, 10^{-8} or 10^{-10}M) at 44 DAS on the length (cm), fresh and dry mass (g) of root of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Root length		Root fresh mass		Root dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	6.66	8.76	1.24	1.86	0.230	0.317
HBL (10^{-6} M)	6.72	11.96	1.25	2.44	0.232	0.424
HBL (10^{-8} M)	6.73	12.87	1.25	2.65	0.233	0.468
HBL (10^{-10} M)	6.72	11.00	1.24	2.34	0.232	0.409
EBL (10^{-6} M)	6.90	12.78	1.26	2.74	0.236	0.449
EBL (10^{-8} M)	6.91	13.99	1.27	2.98	0.237	0.525
EBL (10^{-10} M)	6.89	11.93	1.26	2.49	0.235	0.419
LSD at 5%	1.02	1.67	0.19	0.35	0.036	0.065

Table 23 **Effect of foliar applied HBL or EBL (0, 10^{-6}, 10^{-8} or 10^{-10}M) at 44 DAS on the length (cm), fresh and dry mass (g) of shoot of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Shoot length		Shoot fresh mass		Shoot dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	8.42	13.54	3.09	5.39	0.795	1.356
HBL (10^{-6} M)	8.55	18.75	3.14	7.22	0.814	1.874
HBL (10^{-8} M)	8.56	20.37	3.16	8.11	0.816	2.121
HBL (10^{-10} M)	8.54	17.81	3.11	6.95	0.813	1.806
EBL (10^{-6} M)	8.61	19.84	3.15	7.70	0.829	1.970
EBL (10^{-8} M)	8.62	22.40	3.20	8.87	0.834	2.326
EBL (10^{-10} M)	8.59	18.70	3.15	7.06	0.815	1.820
LSD at 5%	1.33	2.71	0.48	1.19	0.12	0.28

4.4.7 Photosynthetic rate (P_N)

The data presented in table 25 express that the plants, when received foliar spray of brassinosteroids analogues (HBL/EBL) photosynthesized more effectively than the control plants, at both the stages (45 and 60 DAS) of growth. Maximum increase of about 37.6% was recorded in the leaves of plants sprayed with 10^{-8} M of EBL, whereas, by 10^{-8} M of HBL it was about 32.5% at 45 DAS.

4.4.8 Stomatal conductance (g_s)

The values exhibited a progressive increased as the growth progressed from day 45 to 60 DAS (Table 25). Significant increase was recorded in the g_s of plants when given foliar spray of either of the brassinosteroid analogues (HBL/EBL) at the two stages of growth (45 and 60 DAS). 10^{-8}M concentration of HBL/EBL proved to be best over others. EBL was more effective than HBL.

4.4.9 Internal CO_2 concentration (C_i)

The C_i (Table 25) followed a pattern similar to that of g_s. Foliar spray of brassinosteroid analogues (HBL/EBL) significantly increased the C_i. Maximum value for C_i were recorded at 45 DAS by EBL (10^{-8}M) spray, which was about 23.8 % more as compared to control plants.

4.4.10 Water use efficiency (WUE)

The value increased as the age of the plants advanced from day 45 to 60 (Table 26). A significant increase was found in the WUE of tomato plants given foliar application of HBL/EBL (10^{-6}, 10^{-8} or 10^{-10}M) at both the stages of growth (45 or 60 DAS). Out of the two brassinosteroid analogues, EBL (10^{-8} M) generated a better response and enhanced the values of WUE by 60.1% over their control at 60 DAS.

Table 24 **Effect of foliar applied HBL or EBL (0, 10^{-6}, 10^{-8} or 10^{-10}M) at 44 DAS on the leaf area (cm^2), RWC (%) and SPAD chlorophyll of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Leaf area		RWC		SPAD chlorophyll	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	6.00	8.00	76.4	82.1	38.6	45.2
HBL (10^{-6} M)	6.18	9.96	92.8	97.8	54.1	56.6
HBL (10^{-8} M)	6.21	10.63	102.8	104.7	56.0	59.0
HBL (10^{-10} M)	6.18	9.74	88.9	93.2	51.4	54.8
EBL (10^{-6} M)	6.19	10.47	96.5	100.5	55.1	57.8
EBL (10^{-8} M)	6.29	11.72	113.5	111.2	60.6	62.1
EBL (10^{-10} M)	6.20	9.92	90.6	94.6	52.8	55.5
LSD at 5%	0.96	1.52	7.43	7.80	4.58	4.79

Table 25 **Effect of foliar applied HBL or EBL (0, 10^{-6}, 10^{-8} or 10^{-10}M) at 44 DAS on the P_N [μ mol (CO_2) $m^{-2}s^{-1}$], g_s (mol $m^{-2}s^{-1}$) and C_i (ppm) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	P_N		g_s		C_i	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	7.21	9.75	0.060	0.080	323	348
HBL (10^{-6} M)	9.28	11.65	0.090	0.117	373	385
HBL (10^{-8} M)	9.55	12.08	0.097	0.123	389	392
HBL (10^{-10} M)	9.00	11.36	0.083	0.107	360	376
EBL (10^{-6} M)	9.38	11.85	0.093	0.119	380	389
EBL (10^{-8} M)	9.92	12.63	0.105	0.136	400	414
EBL (10^{-10} M)	9.12	11.56	0.084	0.112	370	381
LSD at 5%	0.85	1.07	0.006	0.008	33.6	35.1

4.4.11 Transpiration rate (E)

The transpiration rate in tomato plants expressed a response similar to that of WUE, during the growth period (Table 26). A significant increase in the E was recorded in the plants sprayed with either of the hormone (HBL/EBL). EBL (10^{-6}, 10^{-8} or 10^{-8}M) sprayed leaves showed maximum E at 45 DAS which was 50.7, 65.9 and 45.6% more over the control. A similar trend was followed by the plants at 60 day sampling.

4.4.12 Nitrate reductase (NR) activity

It is evident from table 26 that the plants received foliar spray of brassinosteroid analogues (HBL/EBL) possessed significantly higher NR activity, at both the stages of growth (45 and 60 DAS), as compared to their respective controls. However, the activity of the enzyme increased with the advancement of age (45 to 60 DAS). Brassinosteroids (HBL/EBL) with different concentrations (10^{-6}, 10^{-8} or 10^{-10} M) when sprayed to the foliage improved the activity to a significant level. EBL (10^{-6}, 10^{-8} or 10^{-10}M) was better as compared to HBL and enhanced the activity by 39.2, 65.3 and 34.3% and 34.6, 54.4 and 29.8% over their respective controls 45 and 60 DAS respectively.

4.4.13 Carbonic anhydrase (CA) activity

The activity of CA increased with the age of the plants (Table 27). Brassinosteroid analogues (HBL/EBL), with different concentrations (10^{-6}, 10^{-8} or 10^{-10} M) when applied to the foliage improved the CA activity to a significant level. HBL (10^{-8}M) increased the value of CA activity by 48.23 and 24.68% at 45 and 60 DAS over the control, respectively. Maximum enzyme activity was recorded in the leaves sprayed with EBL (10^{-8}M) and was 58.50% and 35.26% higher CA activity at 45 and 60 DAS, over control.

Table 26 **Effect of foliar applied HBL or EBL (0, 10^{-6}, 10^{-8} or 10^{-10}M) at 44 DAS on the WUE, E (mmolm^{-2}sec^{-1}) and NR activity [n mol NO_2 g^{-1}h^{-1} leaf F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	WUE		E		NR activity	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	0.270	0.309	1.97	2.75	329	375
HBL (10^{-6} M)	0.426	0.420	2.81	3.42	447	493
HBL (10^{-8} M)	0.486	0.440	3.04	3.61	469	511
HBL (10^{-10} M)	0.402	0.399	2.67	3.28	424	470
EBL (10^{-6} M)	0.460	0.418	2.97	3.56	458	505
EBL (10^{-8} M)	0.515	0.495	3.27	3.95	544	579
EBL (10^{-10} M)	0.430	0.402	2.87	3.39	442	487
LSD at 5%	0.038	0.040	0.21	0.26	40.5	44.9

Table 27 **Effect of foliar applied HBL or EBL (0, 10^{-6}, 10^{-8} or 10^{-10}M) at 44 DAS on the CA activity [mol (CO_2) kg^{-1}s^{-1}], POX activity [g^{-1}(F.M.)] and CAT activity [μ mol H_2O_2 decomposed g^{-1} (F.M.)] in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	CA activity		POX		CAT	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	3.40	3.97	10.0	12.5	380	427
HBL (10^{-6} M)	4.81	4.70	12.0	14.5	435	470
HBL (10^{-8} M)	5.04	4.95	14.0	16.6	449	476
HBL (10^{-10} M)	4.58	4.54	11.8	14.1	427	459
EBL (10^{-6} M)	4.89	4.85	13.7	16.0	444	472
EBL (10^{-8} M)	5.39	5.37	15.3	17.3	462	503
EBL (10^{-10} M)	4.75	4.66	12.9	15.2	431	463
LSD at 5%	0.39	0.40	0.72	1.25	39.4	42.4

4.4.14 Peroxidase (POX) activity

The data depicted in table 27 clearly revealed an increase in the activity of POX, as growth progressed from 45 to 60 days and also in response to different concentrations (10^{-6}, 10^{-8} or 10^{-10}M) of brassinosteroids (HBL/EBL). Out of the two brassinosteroid analogues (HBL/EBL), EBL (10^{-8} M) proved better and enhance the POX activity to a maximum level.

4.4.15 Catalase (CAT) activity

The activity of enzyme increased as the growth progressed (45 and 60 DAS). Further increased was also observed in response to hormone. Brassinosteroids (HBL/EBL) at three concentrations (10^{-6}, 10^{-8} or 10^{-10} M) when sprayed to the foliage improved the CAT activity to a significant level. Maximum value was recorded with 10^{-8}M of EBL. EBL excelled in its effect over HBL (Table 27).

4.4.16 Superoxide dismutase (SOD) activity

It is evident from table 28 that the leaves which received foliar spray of different concentrations (10^{-6}, 10^{-8} or 10^{-10}M) of hormone (HBL/EBL), possessed higher SOD activity in the plants, over their respective controls at both the stages of growth (45 and 60 days). The HBL (10^{-8}M) showed 24.7 and 16.5% increase in the enzyme activity over the control at 45 and 60 DAS. EBL (10^{-8}M) showed 33.02 and 23.1% higher values over the non-sprayed control plants at 45 and 60 DAS. EBL is more effective than HBL.

4.4.17 Proline content

The data depicted in table 28 clearly revealed a significant increase in the leaf proline content in response to different concentrations (10^{-6}, 10^{-8} or 10^{-10}M) of brassinosteroids (HBL/EBL). Out of the two brassinosteroid analogues (HBL/EBL),

Table 28 **Effect of foliar applied HBL or EBL (0, 10^{-6}, 10^{-8} or 10^{-10}M) at 44 DAS on the SOD activity (units g^{-1} FM) and proline content (mg g^{-1} F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 days DAS**

	SOD		Proline content	
	45 DAS	60 DAS	45 DAS	60 DAS
Control	109	121	10.0	12.5
HBL (10^{-6} M)	130	139	11.7	13.7
HBL (10^{-8} M)	136	141	12.0	14.0
HBL (10^{-10} M)	125	136	11.4	13.1
EBL (10^{-6} M)	133	140	11.8	13.9
EBL (10^{-8} M)	145	149	12.6	14.5
EBL (10^{-10} M)	128	137	11.5	13.3
LSD at 5%	10.7	11.7	1.11	1.31

EBL (10^{-8} M) proved better. The proline content also increased, as the growth progressed.

Experiment 5

4.5.1 Root and shoot length

It is evident from the table 29-30 that the spray of either of the brassinosteroid analogues (HBL/EBL) to the foliage of the tomato plants raised from the pre-sowing seed soaking in NaCl (50, 100 or 150 mM) significantly favoured shoot and root length of the plant and also nullified the salt stress in a concentration dependent manner. Out of the two brassinsoteroid analogues EBL was found to be more effective and improved the values of salt fed plants in a better way than HBL at 60 DAS.

4.5.2 Fresh and dry mass of root

The plants raised from the seeds given pre-sowing seed soaking NaCl treatment and also sprayed with brassinosteroid analogues (HBL/EBL) improved the fresh and dry mass of root and also neutralized the damaging effect of salt to a limited extent. EBL was a better promoter than HBL (Table 29).

4.5.3 Fresh and dry mass of shoot

The value of fresh and dry mass of shoot increased as the growth proceeded (Table 30). However, the foliar spray of brassinosteroids (HBL/ EBL) to the foliage of plants obtained from the seeds given pre sowing seed soaking NaCl treatment significantly favoured shoot mass and also nullified the stress generated by different concentrations of sodium chloride (50, 100 or 150 mM). Out of the two analogues EBL was found to be more promising and increased the fresh and dry mass of shoot and completely neutralize the ill effect generated by 50 or 100 mM NaCl. Whereas,

Table 29 **Effect of 10^{-8}M of HBL or EBL on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in length (cm), fresh and dry mass (g) of root of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Root length		Root fresh mass		Root dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	6.50	9.31	1.16	1.84	0.227	0.401
NaCl (50 mM)+ HBL	5.99	9.91	0.88	1.95	0.158	0.478
NaCl (100 mM)+ HBL	5.15	9.46	0.74	1.69	0.114	0.445
NaCl (150 mM)+ HBL	4.04	8.12	0.67	1.52	0.071	0.351
NaCl (50 mM)+ EBL	6.14	10.20	0.90	1.99	0.160	0.499
NaCl (100 mM)+ EBL	5.35	9.79	0.76	1.72	0.117	0.460
NaCl (150 mM)+ EBL	4.27	8.43	0.66	1.55	0.074	0.354
LSD at 5%	0.81	1.49	0.11	0.27	0.017	0.069

Table 30 **Effect of 10^{-8}M of HBL or EBL on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in length (cm), fresh and dry mass (g) of shoot of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Shoot length		Shoot fresh mass		Shoot dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	9.32	12.96	3.15	5.40	0.795	1.383
NaCl (50 mM)+ HBL	8.51	13.95	2.85	6.38	0.684	1.659
NaCl (100 mM)+ HBL	7.13	12.98	2.50	5.02	0.586	1.372
NaCl (150 mM)+ HBL	5.35	11.25	2.24	4.60	0.478	1.043
NaCl (50 mM)+ EBL	8.73	14.85	2.90	6.63	0.708	1.728
NaCl (100 mM)+ EBL	7.21	13.77	2.58	5.23	0.599	1.381
NaCl (150 mM)+ EBL	5.63	11.74	2.29	4.77	0.496	1.098
LSD at 5%	1.01	1.88	0.36	0.73	0.089	0.19

the ill effect generated by 150 mM was partially neutralized and improved the values statistically equal to that of control.

4.5.4 Leaf area

Leaf area of the plant increased from day 45 to 60 (Table 31). The values decreased as the concentration of salt was increased. However, brassinosteroid (HBL/EBL) spray at 44 DAS neutralized the damage caused by the salt, at both the stages of growth, in a concentration dependent manner. The ill effect generated by the lowest concentration of salt (50 mM) was completely overcome by either of the brassinosteroids at 60 days sampling only.

4.5.5 Relative water content (RWC)

It is evident from the table 31 that the plants raised from the seeds given pre-sowing soaking NaCl treatment when spray with hormone (HBL/EBL) significantly improved the values of relative water contents and also neutralized the damaging effect of salt stress to a limited extent. Brassinsoteroid (HBL/EBL) completely overcame the ill effect of the lowest concentration of NaCl (50 mM) at both the sampling stages.

4.5.6 SPAD chlorophyll

The value for SPAD chlorophyll content increased as the growth proceeded (Table 31). However, the leaves of the plants that were grown from the seeds given pre-sowing seed soaking NaCl treatment of three different concentrations (50, 100 or 150 mM) along with the foliar spray of brassinosteroids (HBL/EBL) showed gradual decrease in the values of chlorophyll content. The stress caused by the salt (50 mM) was effectively neutralized by the brassinosteroids spray at 60 days sampling. EBL was found to be more promising than HBL.

4.5.7 Photosynthetic rate (P_N)

The values of P_N exhibited an increasing trend with the advancement of the plant age (Table 32). The foliar spray of either of the brassinosteroids (HBL/EBL) on the foliage of plants raised from the seeds given pre sowing soaking salt treatment significantly improved the values of P_N, but in a concentration dependent manner. The ill effect generated by the lowest concentration (50 mM) of salt was completely overcome and of higher concentrations (100 or 150 mM) was partially overcome by EBL or HBL at both the sampling stages. EBL was more effective than HBL.

4.5.8 Stomatal conductance (g_s)

The values of g_s exhibited an increase with increase in the growth period from 45 to 60 days (Table 32). The foliar spray of brassinosteroids (HBL/EBL) to the foliage of plants obtained from the seeds given pre-sowing soaking in salt significantly favoured g_s and also nullified the stress generated by three different concentrations of NaCl (50, 100 or 150 mM). At 60 day stage, plants treated along with the lowest concentration of NaCl and EBL showed maximum values for stomatal conductance over their non-sprayed control plants.

4.5.9 Internal CO_2 concentration (C_i)

The values of C_i increased as the age of the plants advanced (Table 32). However, a significant reduction in C_i was observed in the salinity (100 or 150 mM) treated plants sprayed with either of the brassinosteroid analogues (HBL/EBL). The hormone completely neutralized the damage caused by the lower concentration (50 mM) of salt, however, at higher concentration of salt the effect of hormone was less effective and cannot overcome the damage completely.

Table 31 **Effect of 10^{-8}M of HBL or EBL on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in leaf area (cm^2), RWC (%) and SPAD chlorophyll of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Leaf area		RWC		SPAD chlorophyll	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	5.29	6.62	74.80	87.20	38.8	45.1
NaCl (50 mM)+ HBL	4.18	7.10	85.09	97.10	45.0	51.6
NaCl (100 mM)+ HBL	3.78	6.20	80.00	90.30	39.8	46.0
NaCl (150 mM)+ HBL	3.14	5.38	68.80	77.00	35.3	41.9
NaCl (50 mM)+ EBL	4.32	7.25	86.45	98.26	46.3	52.5
NaCl (100 mM)+ EBL	3.89	6.46	80.58	92.16	40.9	47.4
NaCl (150 mM)+ EBL	3.27	5.58	67.00	73.50	36.1	44.5
LSD at 5%	0.57	0.94	6.15	7.17	3.34	3.84

Table 32 **Effect of 10^{-8}M of HBL or EBL on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in P_N [μ mol (CO_2) $m^{-2}s^{-1}$], g_s (mol $m^{-2}s^{-1}$) and C_i (ppm) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	P_N		g_s		C_i	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	7.50	9.84	0.061	0.082	325	346
NaCl (50 mM)+ HBL	8.56	10.93	0.063	0.083	344	358
NaCl (100 mM)+ HBL	7.60	9.80	0.057	0.078	300	327
NaCl (150 mM)+ HBL	6.25	8.50	0.045	0.063	274	301
NaCl (50 mM)+ EBL	8.84	10.99	0.066	0.087	353	370
NaCl (100 mM)+ EBL	7.84	10.10	0.062	0.082	309	336
NaCl (150 mM)+ EBL	6.45	8.80	0.048	0.066	287	314
LSD at 5%	0.66	0.85	0.005	0.007	29.7	32.3

4.5.10 Water use efficiency (WUE)

The values of WUE increased as the growth progressed from 45 to 60 days (Table 33). The foliar spray of either of the brassinosteroids (HBL/EBL) on the plants raised from the seeds given pre-sowing soaking treatment of NaCl significantly favoured WUE and also nullified the stress to a limited extent. Out of the two brassinosteroids (HBL/EBL), EBL was found to be more effective and improved the values of salt fed plants 36.5% at 50 mM NaCl over their control at 60 DAS.

4.5.11 Transpiration rate (E)

The foliar spray of brassinosteroids (HBL/EBL) to the foliage of plants obtained from the seeds given pre-sowing soaking treatment of salt, significantly improved the E and also nullified the stress generated by NaCl. At 60 day stage of growth, plants treated with lowest concentration of NaCl along with EBL showed maximum response to E and increase the values over their control (Table 33).

4.5.12 Nitrate reductase (NR) activity

The values of NR activity increased as the age of the plants advanced (Table 33). The follow up treatment of the plant hormone brassinosteroids (HBL/EBL) to the foliage of the plant raised from pre-sowing salt treated seeds significantly enhanced the enzyme activity and also nullified the salinity stress (50 or 100 mM). The ill effect generated by the lowest concentration of salt (50 mM) was completely ameliorated by the either of brassinosteroids (HBL /EBL). EBL was more effective than HBL.

4.5.13 Carbonic anhydrase (CA) activity

The values of CA activity increased as the growth progressed (Table 34). Plants raised from pre-sowing treated seeds with NaCl, when applied with foliar spray of either of the brassinosteroids analogues (HBL/EBL) at 44 DAS showed a significant improvement in the CA activity at both the stages of growth. EBL spray

Table 33 **Effect of 10^{-8}M of HBL or EBL on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in WUE, E ($mmolm^{-2}sec^{-1}$) and NR activity [n mol NO_2 $g^{-1}h^{-1}$ leaf F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	WUE		E		NR activity	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	0.257	0.297	1.95	2.70	327	374
NaCl (50 mM)+ HBL	0.341	0.379	2.17	2.93	394	434
NaCl (100 mM)+ HBL	0.291	0.324	1.94	2.63	350	383
NaCl (150 mM)+ HBL	0.259	0.295	1.66	2.18	274	295
NaCl (50 mM)+ EBL	0.351	0.390	2.30	3.06	410	445
NaCl (100 mM)+ EBL	0.299	0.330	1.97	2.67	347	378
NaCl (150 mM)+ EBL	0.267	0.300	1.74	2.30	284	305
LSD at 5%	0.023	0.026	0.18	0.25	28.0	30.1

Table 34 **Effect of 10^{-8}M of HBL or EBL on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in CA activity [mol (CO_2) $kg^{-1}s^{-1}$], POX activity [g^{-1}(F.M.)] and CAT activity [μ mol H_2O_2 decomposed g^{-1} (F.M.)] in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	CA activity		POX		CAT	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	3.56	4.09	10.55	12.45	378	429
NaCl (50 mM)+ HBL	4.13	4.64	16.48	18.53	488	533
NaCl (100 mM)+ HBL	3.76	4.15	17.96	20.65	533	585
NaCl (150 mM)+ HBL	2.98	3.29	20.37	23.14	620	658
NaCl (50 mM)+ EBL	4.34	4.70	17.07	19.28	517	564
NaCl (100 mM)+ EBL	3.66	4.29	19.24	21.65	567	618
NaCl (150 mM)+ EBL	3.13	3.52	21.37	24.14	650	699
LSD at 5%	0.36	0.41	1.54	1.73	43.9	47.9

proved to be more effective than HBL at all salt concentrations and completely ameliorated the ill effect of 50 mM of NaCl at both the sampling stages.

4.5.14 Peroxidase (POX) activity

The POX activity exhibited an increase, as the growth progressed (Table 34). The follow up treatment of the plant brassinosteroid analogues (HBL/EBL) to the plant foliage raised from pre sowing NaCl treated seeds significantly enhanced the POX activity at both the stages of growth. EBL showed maximum response.

4.5.15 Catalase (CAT) activity

The values of leaf CAT activity advanced as the growth progressed from 45 to 60 DAS. The foliar spray of either of the brassinosteroids (HBL/EBL) on the plants raised from the seeds given pre-sowing soaking in NaCl, significantly increased the CAT activity. Out of the two brassinosteroids (HBL/EBL), EBL was found to be more effective than HBL.

4.5.16 Superoxide dismutase (SOD) activity

The activity of SOD increased as the growth of the plant progressed. The plants sprayed with either of brassinosteroid analogue (EBL/HBL) and also received NaCl through seed soaking possess more value for SOD activity at both the sampling stages as compared to control. EBL was more effective than HBL.

4.5.17 Proline content

The level of the endogenous proline content increased as the growth progressed (Table 35). The foliar spray of brassinosteroids (HBL/EBL) to the foliage of the plants obtained from the seeds given pre-sowing soaking treatment in NaCl (50, 100 or 150 mM) significantly improved the value of proline content. Out of the two analogues, EBL was found to be more effective than HBL.

Table 35 **Effect of 10^{-8}M of HBL or EBL on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in SOD activity (units g^{-1} FM) and proline content (mg g^{-1} F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	SOD		Proline content	
	45 DAS	60 DAS	45 DAS	60 DAS
Control	106	122	10.5	12.7
NaCl (50 mM)+ HBL	206	223	16.5	18.2
NaCl (100 mM)+ HBL	225	230	17.9	19.6
NaCl (150 mM)+ HBL	264	265	19.6	12.4
NaCl (50 mM)+ EBL	217	234	17.5	19.8
NaCl (100 mM)+ EBL	242	256	18.8	21.6
NaCl (150 mM)+ EBL	277	286	20.5	24.4
LSD at 5%	20.74	22.37	1.42	1.57

Experiment 6

4.6.1 Root and shoot length

It is evident from the table 36-37 that the spray of either of the brassinosteroid analogues (HBL/EBL) to the foliage of the tomato plants raised from the seeds sown in soil amended with NaCl (2.9, 5.8 or 8.7 mg Kg^{-1}) significantly favoured shoot and root length of the plant and also nullified the salt stress in a concentration dependent manner. Out of the two brassinsoteroid analogues EBL was found to be more effective and improved the values of salt fed plants in a better way than HBL at 60 DAS.

4.6.2 Fresh and dry mass root

It is evident from table 36 that the plants raised from the seeds sown in the soil amended with NaCl (2.9 mg Kg^{-1}) and also sprayed with brassinosteroid analogues (HBL/EBL) improved the fresh and dry mass of root at 60 DAS over the control. EBL was a better promoter than HBL.

4.6.3 Fresh and dry mass of shoot

The value of fresh and dry mass of shoot increased as the growth progressed (Table 37). The foliar spray of brassinosteroids (HBL/ EBL) to the foliage of plants obtained from the soil amended with sodium chloride (2.9 mg Kg^{-1}) significantly increased the shoot mass at 60 DAS over the control. Out of the two analogues, EBL was found to be more effective in neutralizing the ill effect generated by NaCl.

4.6.4 Leaf area

Leaf area of the plant increased from day 45 to 60 (Table 38). The values decreased as the concentration of salt was increased even in the presence of brassinosteroid (HBL/EBL) spray at 45 DAS. However, at 60 days sampling, the leaf area was increased in the plants obtained from the seeds sown in the soil amended

Table 36 **Effect of 10^{-8}M of HBL or EBL on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in length (cm), fresh and dry mass (g) of root of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Root length		Root fresh mass		Root dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	6.14	9.28	1.20	1.90	0.225	0.400
NaCl (2.9 mg Kg^{-1})+ HBL	5.73	10.07	0.99	2.04	0.168	0.500
NaCl (5.8 mg Kg^{-1})+ HBL	5.36	9.70	0.85	1.81	0.139	0.463
NaCl (8.7 mg Kg^{-1})+ HBL	4.61	8.51	0.78	1.66	0.090	0.369
NaCl (2.9 mg Kg^{-1})+ EBL	5.85	10.45	1.04	2.08	0.176	0.531
NaCl (5.8 mg Kg^{-1})+ EBL	5.53	9.94	1.02	1.85	0.143	0.474
NaCl (8.7 mg Kg^{-1})+ EBL	4.81	8.67	0.98	1.70	0.094	0.379
LSD at 5%	0.78	1.35	0.14	0.28	0.020	0.058

Table 37 **Effect of 10^{-8}M of HBL or EBL on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in length (cm), fresh and dry mass (g) of shoot of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Shoot length		Shoot fresh mass		Shoot dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	9.42	12.94	3.20	5.45	0.773	1.394
NaCl (2.9 mg Kg^{-1})+ HBL	8.93	14.32	2.94	6.73	0.656	1.720
NaCl (5.8 mg Kg^{-1})+ HBL	8.25	13.15	2.74	5.40	0.555	1.473
NaCl (8.7 mg Kg^{-1})+ HBL	6.35	11.74	2.32	4.87	0.458	1.005
NaCl (2.9 mg Kg^{-1})+ EBL	9.18	15.09	2.99	7.06	0.661	1.783
NaCl (5.8 mg Kg^{-1})+ EBL	8.74	14.07	2.77	5.55	0.559	1.522
NaCl (8.7 mg Kg^{-1})+ EBL	8.71	12.48	2.34	5.04	0.464	1.097
LSD at 5%	1.28	2.05	0.41	0.82	0.082	0.203

with 2.9 mg Kg^{-1} of NaCl and also received spray of brassinosteroid analogues. EBL was more effective than HBL.

4.6.5 Relative water content (RWC)

It is evident from the table 38 that the plants raised from the seeds sown in the soil amended with 2.9 mg Kg^{-1} of NaCl and also sprayed with brassinosteroids (HBL/EBL) significantly improved the values of RWC as compared to control at 60 DAS. EBL was more effective than HBL.

4.6.6 SPAD chlorophyll

The value for SPAD chlorophyll increased as the growth advanced (Table 38). However, the leaves of the plants that were grown from the seeds sown in soil amended with NaCl (2.9, 5.8 or 8.7 mg Kg^{-1}) along with the foliar spray of brassinosteroids (HBL/EBL) possess more chlorophyll content than control at both the growth stages. EBL was found to be more effective than HBL.

4.6.7 Photosynthetic rate (P_N)

The values of P_N exhibited an increasing trend with the advancement of the plant age (Table 39) irrespective of treatment. The foliar spray of either of the brassinosteroids (HBL/EBL) on the foliage of plants raised from seed sown in soil mixed with NaCl (2.9, 5.8 or 8.7 mg Kg^{-1}) improved the values of P_N. The stress caused by lower concentration of NaCl was completely neutralized where as of higher concentration was partially by both the brassinosteroid analogues at both the sampling stage.

4.6.8 Stomatal conductance (g_s)

The foliar spray of brassinosteroids (HBL/EBL) to the foliage of plants obtained from the seed sown in soil amended with NaCl significantly improved the g_s over their control. At both the stages of growth, plants treated with lowest

Table 38 **Effect of 10^{-8}M of HBL or EBL on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in leaf area (cm^2), RWC (%) and SPAD chlorophyll of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Leaf area		RWC		SPAD chlorophyll	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	5.20	6.59	75.4	88.6	38.1	46.4
NaCl (2.9 mg Kg^{-1})+ HBL	4.48	7.27	89.4	102.2	45.8	54.6
NaCl (5.8 mg Kg^{-1})+ HBL	3.78	6.35	81.9	94.1	40.2	48.0
NaCl (8.7 mg Kg^{-1})+ HBL	3.16	5.65	71.1	80.1	35.9	42.9
NaCl (2.9 mg Kg^{-1})+ EBL	4.53	7.52	93.1	104.2	47.4	56.4
NaCl (5.8 mg Kg^{-1})+ EBL	3.81	6.71	84.0	101.6	42.1	52.7
NaCl (8.7 mg Kg^{-1})+ EBL	3.19	5.68	77.9	81.8	36.6	45.1
LSD at 5%	0.58	0.97	7.19	7.55	3.26	3.97

Table 39 **Effect of 10^{-8}M of HBL or EBL on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in P_N [μ mol (CO_2) $m^{-2}s^{-1}$], g_s (mol $m^{-2}s^{-1}$) and C_i (ppm) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	P_N		g_s		C_i	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	7.54	9.51	0.063	0.081	326	344
NaCl (2.9 mg Kg^{-1})+ HBL	8.80	10.70	0.066	0.090	351	373
NaCl (5.8 mg Kg^{-1})+ HBL	7.73	9.51	0.060	0.081	313	322
NaCl (8.7 mg Kg^{-1})+ HBL	6.71	8.37	0.056	0.073	287	289
NaCl (2.9 mg Kg^{-1})+ EBL	9.11	10.99	0.070	0.095	364	412
NaCl (5.8 mg Kg^{-1})+ EBL	8.13	9.92	0.065	0.088	318	328
NaCl (8.7 mg Kg^{-1})+ EBL	7.00	8.64	0.057	0.072	298	308
LSD at 5%	0.73	0.92	0.006	0.008	28.2	30.8

concentration of NaCl along with EBL showed maximum values for g_s that were 11.11 and 17.2% higher at 45 & 60 day stage respectively, over their non-sprayed control plants (Table 39).

4.6.9 Internal CO_2 concentration (C_i)

The values of C_i increased as the age of the plants advanced (Table 39). C_i was significantly increased in the leaves of the plants obtained from the seeds sown in the soil amended with NaCl (2.9 mg Kg^{-1}) and also received HBL/EBL to their foliage at both the sampling stage. EBL was more effective than HBL.

4.6.10 Water use efficiency (WUE)

The values of WUE increased as the growth progressed from 45 days stage to 60 days (Table 40). The foliar spray of either of the brassinosteroids (HBL/EBL) improved the WUE in the presence of salt over their control. However, if the salt stressed (2.9 mg Kg^{-1}) plants were sprayed with either of the brassinosteroids (HBL/EBL), the WUE of the plants was 39.3 and 43.6% and 24.6 and 33.8% higher at 45 and 60 DAS over the respective control.

4.6.11 Transpiration rate (E)

The foliar spray of brassinosteroids (HBL/EBL) to the foliage of plants improved the E at both the sampling stage even in the presence of salt. However, if the salt stressed (2.9 mg Kg^{-1}) plants also receive HBL/EBL to their foliage the values were 16.16 and 21.21% and 14.33 and 18.28% higher at 45 and 60 DAS over their respective controls. EBL was more effective than HBL.

4.6.12 Nitrate reductase (NR) activity

The values of NR activity increased as the age of the plants advanced (Table 40) and also by the spray of brassinosteroid analogue to the salt stressed plants. The values of NR were significantly higher in the plants grown in the presence of NaCl

(2.9 mg Kg^{-1}) and also received HBL/EBL to their foliage over their control. EBL was more effective than HBL.

4.6.13 Carbonic anhydrase (CA) activity

The activity of CA was significantly higher in the leaves of the plants obtained from the seeds sown in the soil mixed with lower concentration (2.9mg Kg^{-1}) of salt and also received HBL/EBL to their foliage over their control at both the sampling stage. It was 18.0 and 23.1% and 15.25 and 22.25% more over control at 45 and 60 DAS. EBL was more effective than HBL.

4.6.14 Peroxidase (POX) activity

The peroxidase activity exhibited an increase with growth of the plant from 45 to 60 days (Table 41). The activity increased in the presence of NaCl as well as brassinosteroid analogue. The maximum POX activity was recorded in the plants grown in the presence of 8.7 mg $Kg^{-1}$of NaCl and also received spray of brassinosteroids where as, minimum POX activity was recorded in the control plants.

4.6.15 Catalase (CAT) activity

The activity of CAT shows a similar trend as that of POX. The activity increased with the increasing concentration of NaCl and also received spray of brassinosteroid analogues. Out of the two brassinosteroid analogues, EBL was more effective than HBL (Table 41).

4.6.16 Superoxide dismutase (SOD) activity

The activity of SOD increased as the growth progressed from 45 to 60 DAS irrespective of treatment (Table 42). All the treatment significantly increased the SOD activity and maximum activity of SOD was recorded in the plants received 8.7 mg Kg^{-1} of NaCl and brassinosteroid analogues. EBL was more effective than HBL.

Table 40 **Effect of 10^{-8}M of HBL or EBL on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in WUE, E ($mmolm^{-2}sec^{-1}$) and NR activity [n mol NO_2 $g^{-1}h^{-1}$ leaf F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	WUE		E		NR activity	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	0.257	0.293	1.98	2.79	325	376
NaCl (2.9 mg Kg^{-1})+ HBL	0.358	0.365	2.30	3.19	404	457
NaCl (5.8 mg Kg^{-1})+ HBL	0.305	0.338	2.03	2.93	359	403
NaCl (8.7 mg Kg^{-1})+ HBL	0.265	0.306	1.82	2.51	297	331
NaCl (2.9 mg Kg^{-1})+ EBL	0.369	0.392	2.40	3.30	421	475
NaCl (5.8 mg Kg^{-1})+ EBL	0.319	0.350	2.07	2.98	362	407
NaCl (8.7 mg Kg^{-1})+ EBL	0.273	0.320	1.87	2.60	305	341
LSD at 5%	0.023	0.027	0.19	0.26	28.1	31.4

Table 41 **Effect of 10^{-8}M of HBL or EBL on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in CA activity [mol (CO_2) $kg^{-1}s^{-1}$], POX activity [g^{-1}(F.M.)] and CAT activity [μ mol H_2O_2 decomposed g^{-1} (F.M.)] in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	CA activity		POX		CAT	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	3.50	4.00	10.19	12.20	376	430
NaCl (2.9 mg Kg^{-1})+ HBL	4.13	4.61	15.30	17.48	475	527
NaCl (5.8 mg Kg^{-1})+ HBL	3.83	4.28	17.09	19.43	517	572
NaCl (8.7 mg Kg^{-1})+ HBL	3.21	3.52	19.44	22.21	595	651
NaCl (2.9 mg Kg^{-1})+ EBL	4.31	4.89	16.32	18.51	498	548
NaCl (5.8 mg Kg^{-1})+ EBL	3.66	4.78	18.15	20.15	546	590
NaCl (8.7 mg Kg^{-1})+ EBL	3.30	3.62	20.19	23.08	621	688
LSD at 5%	0.33	0.43	1.45	1.66	40.6	45.0

4.6.17 Proline content

The leaf proline content of the plants increased with the advancement in the age of the plants. All the treatment significantly increased the proline content at both the sampling stages. Minimum proline content was recorded in the control plants.

Experiment 7

4.7.1 Root and shoot length

The data depicted in table 43-44 clearly revealed that the length of shoot and root exhibited a progressive increase from day 45 to 60. Pre-sowing seed soaking treatment in NaCl (50, 100 or 150 mM) concentrations and also soaked in SNP favoured elongation, and also overcame the toxic effect of NaCl in tomato plants. SNP along with NaCl (50 mM) induced maximum values for both shoot and root length that were 3.25% and 4.57% higher over their respective controls at 60 DAS.

4.7.2 Fresh and dry mass of root

The fresh and dry mass of root increased in response to the age of the plants (Table 43). Plants raised from the seeds soaked in salt before sowing and also with SNP showed significant response in the root mass. The plants obtained from the seeds soaked in SNP and also soaked in 50 mM of NaCl possess maximum value for fresh and dry mass of root at 60 DAS. It was 1.25 and 7.21% higher over the control.

4.7.3 Fresh and dry mass of shoot

The plants, with the advancement of the age exhibited higher fresh and dry mass of shoot (Table 44). The plants obtained from the seeds pre-treated with highest concentration of NaCl (150 mM) and also soaked in SNP possessed minimum fresh and dry mass of shoot. However, the treatment of SNP as seed pre-sowing soaking improved the fresh and dry mass of shoot in the plant which also received lowest

Table 42 **Effect of 10^{-8}M of HBL or EBL on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in SOD activity (units g^{-1} FM) and proline content (mg g^{-1} F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	SOD		Proline content	
	45 DAS	60 DAS	45 DAS	60 DAS
Control	105	120	10.1	12.2
NaCl (2.9 mg Kg^{-1})+ HBL	194	208	15.4	17.7
NaCl (5.8 mg Kg^{-1})+ HBL	212	232	16.7	18.8
NaCl (8.7 mg Kg^{-1})+ HBL	245	270	18.3	21.7
NaCl (2.9 mg Kg^{-1})+ EBL	205	225	16.6	19.4
NaCl (5.8 mg Kg^{-1})+ EBL	230	247	17.7	20.5
NaCl (8.7 mg Kg^{-1})+ EBL	261	275	19.2	22.7
LSD at 5%	17.44	19.09	1.5	1.81

Table 43 **Effect of pre-sowing seed soaking in 10^{-5}M of sodium nitroprusside (SNP) for 8h on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in length (cm), fresh and dry mass (g) of root of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Root length		Root fresh mass		Root dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	6.19	8.97	1.24	1.60	0.246	0.402
NaCl (50 mM) + SNP	5.60	9.38	1.18	1.62	0.266	0.431
NaCl (100 mM) + SNP	4.66	8.94	1.09	1.44	0.258	0.410
NaCl (150 mM) + SNP	3.72	7.45	0.99	1.30	0.175	0.310
LSD at 5%	0.71	1.37	0.15	0.21	0.037	0.061

Table 44 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in length (cm), fresh and dry mass (g) of shoot of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Shoot length		Shoot fresh mass		Shoot dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	8.76	12.62	3.19	5.73	0.789	1.378
NaCl (50 mM) + SNP	9.23	13.03	3.46	6.01	0.883	1.483
NaCl (100 mM) + SNP	8.19	11.41	3.22	5.52	0.737	1.246
NaCl (150 mM) + SNP	6.95	9.38	2.57	4.49	0.560	0.956
LSD at 5%	1.25	1.76	0.49	0.85	0.105	0.184

concentration of salt. The values were 4.88 and 7.62% higher for fresh and dry mass respectively over the control at 60 DAS.

4.7.4 Leaf area

Leaf area exhibited an increasing trend with the advancement of plant age (Table 45). Tomato plants had significant reduction in the leaf area in response to the NaCl (150 mM) treatment along with pre-sowing seed soaking nitric oxide treatment. Application of SNP along with 50 or 100 mM of NaCl improved the values which were statistically at par to the control at 60 DAS.

4.7.5 Relative water content (RWC)

RWC was relatively higher at 60 DAS than 45 DAS irrespective of the treatment. The stress generated by 50 and 100 mM of NaCl was completely neutralized by the pre-sowing seed soaking treatment of SNP at 60 DAS. Whereas, 150mM of stress was partially neutralized. However, the treatment of SNP was less effective at 45 day sampling.

4.7.6 SPAD chlorophyll

It is evident from table 45 that leaves of the plants that were raised from the seeds given pre-sowing seed soaking treatment initially in NaCl (50, 100 or 150 mM) and later in SNP possessed higher chlorophyll content at 60 DAS compared to 45 DAS.

4.7.7 Photosynthetic rate (P_N)

Leaves of the plant that were raised from the seeds given pre-sowing seed soaking treatment of NaCl (50, 100 or 150 mM) and also soaked in SNP treatment possess P_N at 60 DAS as compared to 45 DAS. The leaves of the plants obtained from the seeds soaked in 50 or 100mM of NaCl and also soaked in 10^{-5}M of SNP photosynthesized at equal rate than that of control. Whereas, the leaves obtained from

Table 45 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in leaf area (cm^2), RWC (%) and SPAD chlorophyll of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Leaf area		RWC		SPAD chlorophyll	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	5.34	7.54	79.90	87.25	38.2	44.4
NaCl (50 mM) + SNP	5.58	7.74	86.01	90.95	42.2	46.9
NaCl (100 mM) + SNP	4.72	6.20	80.80	87.35	38.3	44.4
NaCl (150 mM) + SNP	3.92	5.15	66.3	67.18	31.9	35.2
LSD at 5%	0.70	0.93	6.55	7.15	3.06	3.55

Table 46 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in P_N [μ mol (CO_2) $m^{-2}s^{-1}$], g_s (mol $m^{-2}s^{-1}$) and C_i (ppm) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	P_N		g_s		C_i	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	7.63	9.70	0.060	0.079	336	349
NaCl (50 mM) + SNP	8.28	10.21	0.061	0.080	348	353
NaCl (100 mM) + SNP	7.64	9.71	0.051	0.064	297	295
NaCl (150 mM) + SNP	5.83	7.14	0.040	0.058	254	277
LSD at 5%	0.63	0.80	0.005	0.006	24.9	27.6

the seeds soaked in 150mM of NaCl and also received SNP possess statistically lower P_N than control at both the stages of sampling.

4.7.8 Stomatal conductance (g_s)

It is evident from table 46 that the g_s increased as the age advanced from day 45 onwards. Plant raised from the seeds soaked in 100 or 150mM of NaCl and also soaked in SNP possessed lower g_s as compared to control at both the stages of sampling. However, the leaves of the plants obtained from the seeds soaked in 50mM of NaCl and also soaked in 10^{-5}M of SNP possess statistically equal value for g_s compared to control.

4.7.9 Internal CO_2 concentration (C_i)

The value of C_i was significantly affected by the treatment. The values were comparatively higher at 60 DAS than 45 DAS. The leaves of the plants obtained from the seeds soaked in 50mM of NaCl and SNP possess statistically equal C_i to control. Whereas, the leaves obtained from the seeds soaked in 100 or 150 mM of NaCl and also soaked in SNP possessed significantly lower values of C_i as compared to control at both the stages of sampling.

4.7.10 Water use efficiency (WUE)

The WUE increased as the growth progressed from 45 to 60 DAS (Table 47). The values were significantly lower in the leaves of the plant raised from the seeds soaked in NaCl (150 mM) and also soaked in SNP at both the stages of growth. Whereas, the seeds soaked in 100 mM of NaCl and also that of SNP possess statistically equal value as compared to control at both the stages of sampling. However, the leaves of the plants raised from the seeds soaked in 50 mM of NaCl and also that of SNP possess significantly higher WUE over the control.

4.7.11 Transpiration rate (E)

It is evident from table 47 that E was relatively higher at 60 DAS as compared to 45 DAS. The values obtained in the plants raised from the seeds soaked in 50 mM NaCl and also soaked in SNP was statistically at par to the control. Whereas, soaking in higher concentration of salt (100 or 150 mM) alongwith SNP shows statistically lower value of E as compared to control at both the stages of sampling.

4.7.12 Nitrate reductase (NR) activity

The activity of the enzyme increased as the growth progressed from 45 to 60 DAS (Table 47). Plants raised from the seeds pre-treated with salt along with follow up treatment with nitric oxide (SNP) possessed significantly different response with three concentrations of salt (50, 100 or 150 mM). The values of NR was significantly higher in leaves of the plants raised from the seeds soaked in 50 mM of NaCl along with SNP over their control at both the stages of sampling and were increased by 13.23% at 45 DAS and 8.39% at 60 DAS over their control.

4.7.13 Carbonic anhydrase (CA) activity

The leaves of the plants that were raised from the seeds given pre-sowing seed soaking treatment with salt (50 or 100 mM NaCl) along with SNP possessed statistically equal value for CA activity to their respective control at both the stages of sampling (Table 48). Whereas, the CA activity was significantly lower in the leaves of the plants raised from the seeds soaked in 150 mM of NaCl along with 10^{-5} M of SNP over their control.

4.7.14 Peroxidase (POX) activity

The activity of POX increased as the growth progressed from 45 to 60 DAS irrespective of the treatment. The maximum POX activity was recorded in the plants obtained from the seeds soaked in 150 mM of NaCl and also soaked in 10^{-5} M of

Table 47 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in WUE, E ($mmolm^{-2}sec^{-1}$) and NR activity [n mol NO_2 $g^{-1}h^{-1}$ leaf F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	WUE		E		NR activity	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	0.250	0.290	1.92	2.67	325	381
NaCl (50 mM) + SNP	0.309	0.346	2.03	2.74	368	413
NaCl (100 mM) + SNP	0.258	0.294	1.71	2.41	338	388
NaCl (150 mM) + SNP	0.225	0.244	1.59	2.16	247	280
LSD at 5%	0.024	0.028	0.14	0.21	31.0	36.3

Table 48 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in CA activity [mol (CO_2) $kg^{-1}s^{-1}$], POX activity [g^{-1}(F.M.)] and CAT activity [μ mol H_2O_2 decomposed g^{-1} (F.M.)] in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	CA activity		POX		CAT	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	3.43	4.10	9.82	11.59	378	431
NaCl (50 mM) + SNP	3.82	4.36	12.33	13.47	439	480
NaCl (100 mM) + SNP	3.51	4.12	14.98	16.64	493	519
NaCl (150 mM) + SNP	2.65	3.00	17.93	18.56	561	593
LSD at 5%	0.33	0.39	1.06	1.16	39.4	43.2

SNP, whereas, minimum POX activity was recorded in the control plants at both the growth stages (Table 48).

4.7.15 Catalase (CAT) activity

The activity of CAT also followed a similar pattern as that of POX at both the stages of growth. The maximum increases was noted in the leaves of the plants obtained from the seeds soaked in 150 mM of NaCl and also in that of SNP, which were about 48.41% and 37.59% higher over that of control at 45 and 60 DAS respectively.

4.7.16 Superoxide dismutase (SOD) activity

The activity of SOD increased with the advancement of the age of the plants from 45 to 60 DAS. The minimum SOD was recorded in the control plants and maximum at higher concentration of NaCl along with SNP.

4.7.17 Proline content

Leaf proline content increased irrespective of the treatment. It is also increased with the advancement of the age of the plants. The maximum level of proline was recorded in the leaves of the plants obtained from the seeds soaked in 150 mM of NaCl and also received SNP, whereas, minimum values were noted in the control plants.

Experiment 8

4.8.1 Root and shoot length

The data depicted in table 50-51 clearly revealed that the length of root and shoot exhibited a progressive increase from day 45 to 60. Length of root and shoot were significantly affected by the treatment. The maximum length of root and shoot were recorded in the plants obtained from the seeds soaked in 10^{-5} M of SNP and grown in the soil amended with 2.9 mg Kg^{-1} of NaCl at both the sampling stages

Table 49 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through seed soaking; 50, 100 or 150 mM for 8 h) induced changes in SOD activity (units g^{-1} FM) and proline content (mg g^{-1} F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	SOD		Proline content	
	45 DAS	60 DAS	45 DAS	60 DAS
Control	110	117	12.2	13.3
NaCl (50 mM) + SNP	160	155	15.9	16.5
NaCl (100 mM) + SNP	185	179	18.5	18.9
NaCl (150 mM) + SNP	204	208	21.8	22.2
LSD at 5%	13.6	13.8	1.53	1.55

which were statistically at par to the values obtained in the control plants as well as grown in the soil amended with 5.8 mg Kg^{-1} of NaCl.

4.8.2 Fresh and dry mass of root

The fresh and dry mass of root increased in response to the age of the plants (Table 50). Plants raised from the seeds soaked in 10^{-5} M of SNP and grown in the soil amended with three different concentrations of NaCl (2.9, 5.9 or 8.7 mg Kg^{-1}) showed significant response in the root mass at both the sampling stages. The minimum values for both parameters were recorded in the plants obtained from the seeds soaked in SNP and grown in the soil amended with higher concentration of the salt.

4.8.3 Fresh and dry mass of shoot

As the growth progressed, both fresh and dry mass increased irrespective of the treatment. The maximum values were recorded in the plants obtained from the seeds soaked in SNP and grown in soil amended with lowest concentration (2.9 mg Kg^{-1}) of NaCl, which were statistically at par to that of control at both the stages of growth (Table 51)

4.8.4 Leaf area

Leaf area also followed a similar pattern as that of length, fresh and dry mass of root and shoot. Leaf area again increased with the advancement of the age of the plant (Table 52). The maximum leaf area was recorded in the plants obtained from the seeds soaked in 10^{-5} M of SNP and grown in the soil amended with 2.9 mg Kg^{-1} of NaCl, which were statistically equal to that of control and significantly higher over the plants obtained from the plants grown in soil amended with highest concentration of NaCl.

Table 50 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in length (cm), fresh and dry mass (g) of root of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Root length		Root fresh mass		Root dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	6.27	9.08	1.18	1.71	0.260	0.389
NaCl (2.9 mg Kg^{-1}) + SNP	6.62	9.40	1.24	1.77	0.295	0.431
NaCl (5.8 mg Kg^{-1}) + SNP	6.34	9.10	1.08	1.49	0.281	0.409
NaCl (8.7 mg Kg^{-1}) + SNP	5.39	7.48	0.99	1.39	0.185	0.249
LSD at 5%	0.90	1.37	0.16	0.22	0.037	0.055

Table 51 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in length (cm), fresh and dry mass (g) of shoot of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Shoot length		Shoot fresh mass		Shoot dry mass	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	9.24	12.84	3.06	5.50	0.742	1.351
NaCl (2.9 mg Kg^{-1}) + SNP	9.82	13.27	3.50	6.02	0.851	1.511
NaCl (5.8 mg Kg^{-1}) + SNP	9.19	12.00	2.93	5.00	0.759	1.352
NaCl (8.7 mg Kg^{-1}) + SNP	7.99	10.04	2.60	4.49	0.516	0.914
LSD at 5%	1.33	1.81	0.42	0.71	0.11	0.19

4.8.5 Relative water content (RWC)

It was observed that RWC of the plants increased as the growth of the plant progressed from 45 to 60 DAS (Table 52). However, the RWC of the leaves of the plants obtained from the seeds soaked in 10^{-5} M of SNP and grown in the soil amended with 2.9 mg Kg^{-1} of NaCl is increased to a level which was statistically equal to that of control. Whereas, the plants obtained from soil mixed with highest concentration of salt (8.7 mg Kg^{-1} of NaCl) possess lowest RWC.

4.8.6 SPAD chlorophyll

The values of SPAD increased, as the growth progressed from 45 to 60 DAS (Table 52). The maximum value was recorded in the plants obtained from the seeds soaked in SNP and grown in the soil amended with a lowest concentration of NaCl, which was significantly higher over the control at 45 DAS and statistically at par to the control at 60 DAS.

4.8.7 Photosynthetic rate (P_N)

Leaves of the plant that were raised from the seeds given pre-sowing seed soaking SNP treatment and sown in soil applied NaCl (8.7 mg Kg^{-1}) photosynthesized at a slower rate but when grown in lowest concentration (2.9 mg Kg^{-1}) of salt, photosynthesized more than the control plants (Table 53). The lowest P_N was recorded in the leaves of the plants grown in the soil amended with highest concentration of the salt (8.7 mg Kg^{-1}).

4.8.8 Stomatal conductance (g_s)

It is evident from table 53 that the g_s increased as the age advanced from day 45 onwards. Plant raised from the seeds given pre-sowing seed soaking treatment in SNP and sown in soil mixed with NaCl (8.7 mg Kg^{-1}) possessed lowest values of g_s at both the stages of growth. The toxic effect generated by the salt was more pronounced

Table 52 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in leaf area (cm^2), RWC (%) and SPAD chlorophyll of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	Leaf area		RWC		SPAD chlorophyll	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	5.41	7.00	76.20	84.30	38.5	46.9
NaCl (2.9 mg Kg^{-1}) + SNP	5.86	7.37	84.49	90.74	43.6	50.8
NaCl (5.8 mg Kg^{-1}) + SNP	5.05	6.18	80.53	86.34	39.8	47.4
NaCl (8.7 mg Kg^{-1}) + SNP	4.38	5.29	69.37	72.83	34.7	39.4
LSD at 5%	0.76	0.96	7.16	8.26	3.75	4.37

Table 53 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in P_N [μ mol (CO_2) $m^{-2}s^{-1}$], g_s (mol $m^{-2}s^{-1}$) C_i (ppm) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	P_N		g_s		C_i	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	7.63	9.80	0.064	0.082	324	348
NaCl (2.9 mg Kg^{-1}) + SNP	8.35	10.41	0.065	0.083	339	357
NaCl (5.8 mg Kg^{-1}) + SNP	7.14	8.78	0.058	0.071	296	308
NaCl (8.7 mg Kg^{-1}) + SNP	6.72	8.52	0.053	0.064	276	288
LSD at 5%	0.72	0.83	0.005	0.006	26.9	28.8

at highest salt concentration (8.7 mg Kg^{-1}). However, the leaves of the plants obtained from the seeds soaked in SNP and grown in the soil amended with lowest concentration (2.9 mg Kg^{-1}) of salt possess the highest value for g_s at both the sampling stage which was statistically equal to that of control.

4.8.9 Internal CO_2 concentration (C_i)

It is noted from the table 53 that the C_i in the leaves increased as the growth of the plants progressed from day 45 to 60. The maximum C_i was recorded in the plants grown in the soil amended with lowest concentration (2.9 mg Kg^{-1}) of salt which was statistically equal to that of control. Whereas, minimum C_i was noted at highest concentration of salt along with 10^{-5} M of SNP.

4.8.10 Water use efficiency (WUE)

WUE increased as the growth progressed from 45 to 60 DAS (Table 54). The values were significantly lower in the leaves of the plant raised from the salt (8.7 mg Kg^{-1}) fed soil along with SNP treatment at both the stages of growth. However, the values were significantly higher in the leaves of the plants raised from the soil amended with salt (2.9 mg Kg^{-1}) along with pre-sowing seed soaking in SNP at both the stages of growth.

4.8.11 Transpiration rate (E)

It is evident from table 54 that the plants raised from the seeds given pre-sowing seed soaking treatment in SNP and then sown in soil mixed with NaCl (8.7 mg Kg^{-1}) transpired at a slower rate than their control plants at both the stages of sampling. However, significantly higher E was recorded in the plants obtained from the pre-sowing seed soaking in SNP and grown in the soil amended with lowest concentration of salt over their control at both the stages of sampling and were 10.42% and 7.14% higher at 45 and 60 DAS respectively over control.

4.8.12 Nitrate reductase (NR) activity

The activity of the enzyme increased as the growth progressed from 45 to 60 DAS (Table 54). Plants raised from the seeds pre-sowing seed soaking with SNP and sown in soil amended NaCl (2.9, 5.8 or 8.7 mg Kg^{-1}) possessed significantly different response. The activity of NR was significantly higher in the leaves of the plants raised from the pre-sowing seed soaking in SNP and grown in soil amended with 2.9 mg Kg^{-1} of NaCl over the control. Whereas, the activity of leaves of the plants grown in soil amended with 5.8 mg Kg^{-1} of NaCl was statistically at par to the control.

4.8.13 Carbonic anhydrase (CA) activity

The activity of CA increased with the advancement of the age of the plant. The maximum activity was recorded in the plants raised from the seeds soaked in SNP and grown in the soil mixed with 2.9 mg Kg^{-1} of NaCl which was significantly higher over their control at both the stages of sampling. However, the activity of CA in the leaves of the plants raised from the seeds pre-soaked in the SNP and sown in the soil amended with 5.8 mg Kg^{-1} of NaCl was statistically equal to that of control (Table 55).

4.8.14 Peroxidase (POX) activity

It is evident from the table 55 that POX increased as the growth progressed from 45 to 60 DAS. The maximum POX was recorded in the leaves of the plant raised from the pre-sowing seed soaking in SNP and grown in the soil amended with 8.7 mg Kg^{-1} of NaCl at both the stages of sampling. Whereas, minimum value in control plants.

4.8.15 Catalase (CAT) activity

The activity of CAT increased as the growth of the plant progressed from 45 to 60 DAS. All the treatment significantly improved the CAT activity. The maximum

Table 54 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in WUE, E ($mmolm^{-2}sec^{-1}$) and NR activity [n mol NO_2 $g^{-1}h^{-1}$ leaf F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	WUE		E		NR activity	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	0.251	0.292	1.92	2.80	321	368
NaCl (2.9 mg Kg^{-1}) + SNP	0.322	0.354	2.12	3.00	359	408
NaCl (5.8 mg Kg^{-1}) + SNP	0.266	0.298	1.85	2.57	305	329
NaCl (8.7 mg Kg^{-1}) + SNP	0.229	0.246	1.68	2.39	276	299
LSD at 5%	0.024	0.027	0.15	0.21	28.6	29.2

Table 55 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in CA activity [mol (CO_2) $kg^{-1}s^{-1}$], POX activity [g^{-1}(F.M.)] and CAT activity [μ mol H_2O_2 decomposed g^{-1} (F.M.)] in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	CA activity		POX		CAT	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	3.47	4.12	10.11	12.30	374	432
NaCl (2.9 mg Kg^{-1}) + SNP	3.85	4.46	12.57	14.46	430	482
NaCl (5.8 mg Kg^{-1}) + SNP	3.27	3.68	14.40	16.41	480	517
NaCl (8.7 mg Kg^{-1}) + SNP	3.02	3.31	16.56	18.75	517	550
LSD at 5%	0.26	0.29	1.06	1.19	40.2	43.3

CAT activity was noted in the leaves of the plants obtained from the seeds soaked in SNP and sown in the soil mixed with 8.7 mg Kg^{-1} of NaCl at 45 and 60 DAS. The minimum CAT activity was recorded in the control plants.

4.8.16 Superoxide dismutase (SOD) activity

The activity of SOD was increased irrespective of the treatment and sampling days. The maximum SOD activity was noted in the plants grown in the soil amended with 8.7 mg Kg^{-1} of NaCl and these seeds also received seed soaking treatment of SNP at the time of sowing. Whereas, minimum SOD activity was recorded in the control plants.

4.8.17 Proline content

As the growth of the plant progressed from 45 to 60 DAS, the content of leaf proline increased. The leaves of the plants raised from the seeds soaked in SNP and grown in soil amended with highest concentration of salt (8.7 mg Kg^{-1}) possessed maximum proline content at both the stages of sampling. However, minimum value was recorded in the control plants.

Table 56 **Effect of pre-sowing seed soaking in 10^{-5}M of SNP for 8h on salinity (through soil; 2.9, 5.8 or 8.7 mg Kg^{-1}) induced changes in SOD activity (units g^{-1} FM) and proline content (mg g^{-1} F.M.) in the leaves of tomato (*Lycopersicon esculentum* L. cv. K-21) plants at 45 and 60 DAS**

	SOD		Proline content	
	45 DAS	60 DAS	45 DAS	60 DAS
Control	108	118	11.3	12.9
NaCl (2.9 mg Kg^{-1}) + SNP	126	130	13.4	14.7
NaCl (5.8 mg Kg^{-1}) + SNP	142	147	15.2	16.5
NaCl (8.7 mg Kg^{-1}) + SNP	181	184	18.8	19.7
LSD at 5%	12.1	13.0	1.28	1.35

CHAPTER - 5

DISCUSSION

Out of the genomic information stored in a cell for the synthesis of proteins, needed in the whole life span of the plant to regulate various activities, only a limited portion of it is expressed at any given time. Each set of these proteins (enzymes) is specifically recognized to regulate a definite process of physiochemical expressions, going on in the cell. However, there are forces that play a significant role in selecting the pattern of the expression/derepression of these genes. The important ones are light (Thompson and White, 1991), pollutants/elicitors/phytotoxins (Royals *et al.*, 1992), phytohormones (Cleland, 1999) and also the electrical signals and small biomolecuels generated while establishing communication between the cells (Robards and Lucas, 1990).

Out of the above mentioned forces salinity, nitric oxide (NO) and brassinosteroids (BRs) were selected for the present study. Soil salinity is currently one of the major environmental problems that pose a severe threat to the growth of plants and their productivity. The sufficient volume of literature on the effect of salinity on diverse plant tissues and cells is present although the mechanism involved in its toxicity is still not completely understood.

In recent years NO has emerged as an important endogenous signalling molecule in plants that mediates responses to abiotic and biotic stresses. NO is reported to be involved in the responses to drought stress (Haramaty and Leshem, 1997; Leshem, 1996; Uchida *et al.*, 2002), heat stress (Lesham *et al.*, 1998), disease resistance (Delledonne *et al.*, 1998; Palverari *et al.*, 2003), apoptosis (Pedreso *et al.*, 2000) and formation of lateral roots (Correa-Aragunde *et al.*, 2006). NO may also enhance salt tolerance in plants by activating the expression of plasma membrane

Na^+/H^+ antiporter gene and H^+ ATPase genes that are required for Na^+ homeostasis and K^+ acquisition (Qiao and Fan, 2008).

On the other hand BRs are also a new class of phytohormones that play a vital role in growth by activating cell division, elongation and their proliferation (Arteca, 1997). Moreover, BRs also elicit a positive shift in the responsiveness of the plants (Mandava *et al.*, 1981; Mandava, 1988; Cutler, 1991; Yopp *et al.*, 1981 and Khripach *et al.*, 2000).

In the present study the salt fed through seed or soil caused a significant damage to the plants that is expressed in terms of reduced growth (length of shoot and root, their fresh and dry mass and leaf area, Tables 1-3, 8-10). This supports our earlier observations in *Brassica jucnea* (Hayat *et al.*, 2006) and in *Cicer arietinum* (Ali *et al.*, 2007). Kleinkopf and Wallace (1974) proposed that growth inhibition under salinity could be partly due to shortage of energy because process involved in transport of salts and repair of salt damage exerted on membrane or proteins are energy consuming. This result agrees with Ghoulam *et al.* (2002), who showed a marked reduction in growth parameters of sugarbeet plants under salt stress (Plate IVB).

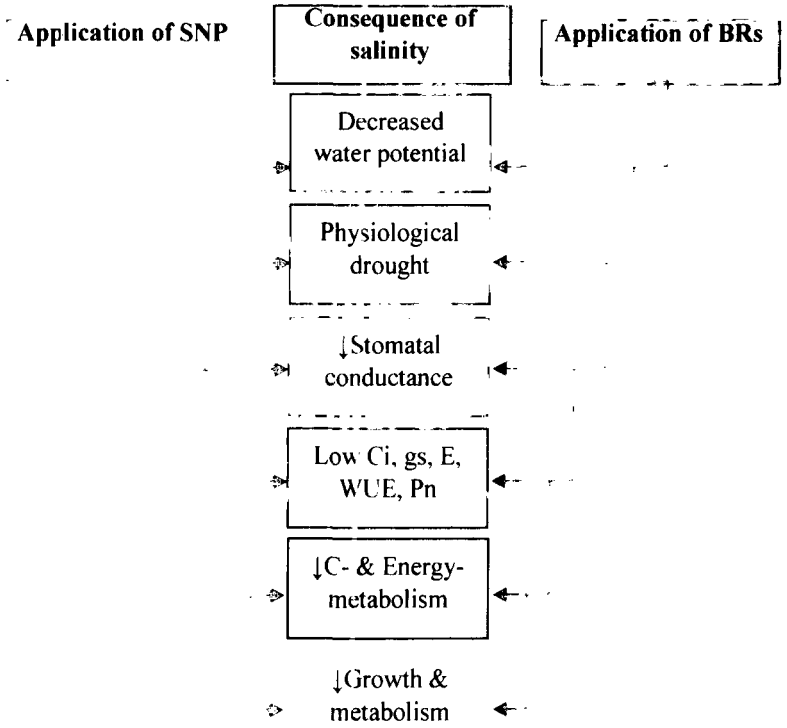
However, the plants resulting from the seeds pre-treated with 10^{-5} M SNP (Sodium nitro-prusside, a NO donor compound) showed significantly higher growth (length, fresh and dry mass of roots and shoots and leaf area) when compared with rest of the concentrations and control (Tables 15-17). This is because SNP possibly favours the activity of exo- and endo-β-D-glucanase in the cell wall (Terasaki *et al.*, 2001) which has been further verified by using NO deficient mutants, where enzyme activity decreased and growth remained restricted (Salgado *et al.*, 2010) The glycosidic linkage between glucose units within the cell wall is broken by these

This drives growth by increasing internal turgor pressure, which could be generated by an increase in RWC (Table 17). Similar observations have also been reported earlier in tomato (Hayat *et al.*, 2010b). NO may serve as a signal molecule in growth and development of plants (Creus *et al.*, 2005; Hu *et al.*, 2005). However, in response to salt stress, NO caused protection against salt stress in young rice seedling (Uchida *et al.*, 2002) and maize seedlings (Zhang *et al.*, 2004). A key factor limiting plant growth is excessive Na^+, a harmful mineral element, not required by most plants. High Na^+ content in the tissue is often considered as the most critical factor responsible for salt toxicity. A possible survival strategy of plants under saline conditions was to sequester absorbed Na^+ in the roots (Plate I).

The application of BRs (EBL or HBL) significantly increased growth characteristics (length, fresh and dry mass of roots and shoots, and leaf area) of the plants (Tables 22-24). The stimulatory effect of BRs on plant growth is mediated through the regulation of gene expression (Felner, 2003). BRs activate the BRU1 and TCH4 genes encoding xyloglucan endotransglycoxylase (XET) and expansins (Cosgrove, 1997). These enzymes are responsible for cell wall loosening. Moreover, BRs affect the RWC (Table 24). The higher RWC builds up a hydrostatic pressure against cell wall, resulting in its loosening. Simultaneously, BRs also maintain a healthy metabolic state in the plants as a whole (Ali *et al.*, 2006), which fulfils the demand for additional material needed for the growth of the cell wall as well as that of the plant as a whole (Table 24, Plate IVA).

Salinity decreases chlorophyll content through an inhibition of chlorophyll synthesis or an acceleration of its degradation (Reddy and Vora, 1986). The chlorophyll content of tomato plants decreased with increasing NaCl concentration. Therefore, a cumulative effect may be generated and expressed in the form of a

A.



B.

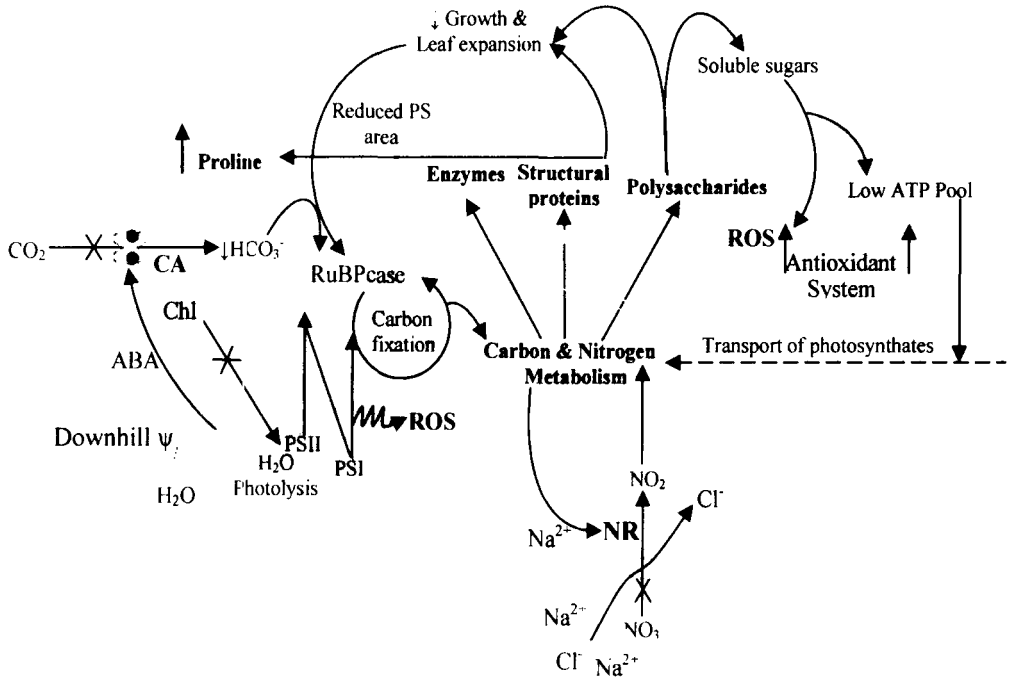


Plate IV: A. Hierarchical consequence of salinity on plant metabolism and the effects of application of BR/SNP.

B. Metabolic processes are linked within a cell to sense sequential alteration of activity under saline conditions

decrease in SPAD chlorophyll values (Tables 3, 10; Plate IVB). This is in conformity with Zhao *et al.* (2007). This damaging effect of salt was overcome in the plants given a follow-up treatment with either BRs (EBL/HBL) (Tables 31, 38) or SNP (Tables 45, 52).

NO increased chlorophyll content in pea leaves particularly in guard cells (Leshem, 1996). This positive effect of NO on chlorophyll retention may reflect NO effects on iron availability (Graziano *et al.*, 2002). BRs fed to the non-stressed plants significantly increased the pigment contents (Table 24) that supports the findings of others (Braun and Wild, 1984; Bhatia and Kaur, 1997; Hayat *et al.*, 2000, 2001; Fariduddin *et al.*, 2003; Yu *et al.*, 2004; Ali *et al.*, 2006, 2007). The reason that sounds best in defending the said observation is possibly the BL-induced impact on transcription and/or translation (Khripach *et al.*, 2003) by involving the expression of specific genes responsible for the synthesis of the enzymes that determine chlorophyll biosynthesis.

Carbonic anhydrase (CA) is the enzyme which catalyzes the reversible hydration of CO_2 and maintains its constant supply to RuBPCase, at the level of the grana of the chloroplast (Majeau and Coleman, 1994; Price *et al.*, 1994). Otherwise at ambient concentration of inorganic carbon, the activity of RuBPCase gets restricted (Majeau and Coleman, 1994; Plate III, IVB). Moreover, CA is also hypothesized to be involved in photosynthetic electron transport system (Stemler, 1997) and in maintaining chloroplast pH during rapid changes in light intensity (Reed and Graham, 1981). The reported decrease in the activity of CA (Tables 6, 13) by NaCl is in agreement with Ali *et al.* (2007). The activity of CA is largely determined by photon flux density, concentration of CO_2, the availability of Zn (Tiwari *et al.*, 2005) and the genetic expression (Kin *et al.*, 1994). A decline in the activity of CA could have been

genetic expression (Kin *et al.*, 1994). A decline in the activity of CA could have been the result of the effect of the inactivation of rubisco (Soussi *et al.*, 1998) which sequentially reduces photosynthetic carbon metabolism, leaf chlorophyll content and photosynthetic efficiency (Seeman and Critihley, 1985; Plate IVB). NO can also regulate stomatal closure by modulating ion channels and Ca^{2+} level in guard cells (Lamattina *et al.*, 2003) and hence 10^{-5} M SNP increased the activity of CA (Table 20) in control as well as in salinity stressed plants (Tables 48, 55). However, higher concentrations of SNP produce a decline in the activity of CA in tomato plants (Table 20). The treatment with BRs, alone or as a follow up treatment to the NaCl stressed seedling elevated the level of CA by possibly speeding up the assimilation of CO_2 (Yu *et al.*, 2004) through the expression of specific genes (Khripach *et al.*, 1999). Moreover, BR signalling is known to be involved independently in the transcription and/or translation of numerous genes including those of transcription factors (Nemhauser *et al.*, 2004) that may positively effect the activity of CA. These results are also in conformity with Hayat *et al.* (2010b) in tomato and Swamy and Rao (2009) in *Pelargonium graveolens*.

The plants exposed to salt stress exhibit a slow down of the photosynthetic rate (P_N) that was accomplished by a significant decrease in stomatal conductance (g_s), internal CO_2 concentration (C_i) water use efficiency (WUE) and transpiration rate (E) (Tables 4-5, 11-12). As mentioned earlier NaCl brings about the closure of stomata by decreasing the partial pressure of CO_2 in the stroma (Iyenger and Reddy, 1996) this becomes the direct cause for the observed loss of g_s, C_i and E. A cumulative effect of all these altered processes leads to a decrease in P_N (Tables 4, 11). The other possible reasons for this decrease in the P_N under the influence of salinity are enhanced senescence and changes of enzymatic activities induced by a

shift in cytoplasmic structure and negative feedback by reduced sink activity (Iyenger and Reddy, 1996) and transport of photosynthates. Low CO_2 decreases the carbon reduction metabolism and hence the translation of proteins/enzymes which further decreases the SPAD chlorophyll value (Tables 3,10). The fall in the activity of CA (Tables 6, 13) is an additional cause of lower P_N (Tables 4, 11; Plate IVB). BRs (HBL/EBL) or NO (10^{-5} M SNP) improved all the photosynthetic attributes (Tables 18-19, 25-26). BRs are also known to have a positive impact on RuBPcase activity (Braun and Wild, 1984), a key enzyme in photosynthetic carbon fixation. Moreover, higher RuBPcase activity, in the treated leaves, may be due to increase in CA activity (Table 27). Higher P_N and related attributes in response to BRs under differential abiotic stresses have also been reported by others (Ali *et al.*, 2008a, b; Fariduddin *et al.*, 2009). This may be the potential reason for improving P_N in the plants supplemented with BRs (Table 25 and Plate IVA). On the other hand 10^{-5} M of SNP (alone or as a follow up treatment to salinity) increased all the photosynthetic attributes (Tables 18-19, 46-47, 53-54 and Plate IVA) to a level less than the response generated by either of the BRs (HBL/EBL). Higher concentrations of SNP, however, supposed to mediate PCD (Plate II).

The activity of nitrate reductase (NR) enzyme exhibited a significant decline in response to sodium chloride (NaCl) treatments (Tables 5, 12). It could be an expression of stress induced enzyme inhibition and/or metabolic dysfunction (Hopkins, 1995). The process of nitrate reduction depends on the three main factors (a) substrate (NO_3) level in the cytoplasm, (b) the level of functional nitrate reductase (NR) and/or (c) the activity level of functional NR. Each of these processes is, directly or indirectly depends on the metabolic sensors and/or signal transducers (Campbell, 1999). Moreover, the major rate limiting step in the whole process of nitrate reduction

NR. Salinity retards the uptake of nitrate (Aslam *et al.*, 1984) which is the substrate cum inducer of NR (Solomonson and Barber, 1990) that may have caused in a decline in the activity of NR (Plate III, IVB). However, 10^{-5} M of SNP (NO) acted as an antioxidant and thus played a protective role (Beligni and Lamattina, 1999). NR activity was stimulated by NO, induced by post translational regulatory pathway (Jin *et al.*, 2009). The NR activity could be markedly enhanced by NAA (an analogue of IAA) in chicory (Vuylsteker *et al.* 1998), by cytokinin benzyladenice in *Arabidopsis* (Yu *et al.*, 2001), and by salicylic acid in maize (Jain and Srivastava, 1981). The NAA has also been reported to significantly increase the NR activity in Chinese cabbage, but this stimulating effect could be reversed by the addition of cPTIO. How these chemical compounds regulate NR, however, is poorly understood. Noticeably, the effects of these chemicals are all related to the NO generation (Tun *et al.*, 2001; García-Mata and Lamattina 2002; Pagnussat *et al.* 2004). It has been reported that electron transfer from haem to molybdenum is the rate-limiting step in the NR-catalysed nitrate reduction (Kay and Barber 1986). Stimulating effect of NO on NR activity might be due to an enhancement of electron transfer from haem to nitrate through activating the haem and molybdenum centers in the NR.

However, the application of BR alone or as a follow up treatment after exposing to salinity increased the activity of NR that could be an expression of the impact of BRs on translation and/or transcription (Khripach *et al.*, 2003). Another possible reason may be the involvement of BRs in increasing the concentration of NO_3 by acting at the level of the membrane (Mai *et al.*, 1989).

In a natural course, the plants exposed to stress generate a large quantity of reactive oxygen species (ROS) (Schutzendubell and Polle, 2002) that may oxidize proteins, lipids and nucleic acids resulting in the abnormalities at the level of the cell

proteins, lipids and nucleic acids resulting in the abnormalities at the level of the cell (di Toppi and Gabrielli, 1999). However, the plants are capable to counter successfully such stress conditions by including the synthesis of antioxidant metabolites (ascorbate, glutathione, tocopherol and proline) and the enzymes [superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathione reductase (GR)] (Schutzendubel and Polle, 2002). These provide additional power of resistance to neutralize the toxic effects of the stress generated through ROS (Apel and Hirt, 2004) such as superoxide radical, hydroxyl ions and H_2O_2. Therefore, the increase in the level of POX, CAT and SOD (Tables 6-7, 13-14) and that of proline (Tables 7, 14) due to salinity stress was natural (Plate IVB). However, the remarkable thing that emerged in the present investigation is that both BRs (EBL or HBL) and NO alone or as a follow up treatment after feeding NaCl through seed or amended in soil increased the values of proline and POX, CAT and SOD. It is well established that BRs have a direct role in the amelioration of oxidative stress (Bajguz and Hayat, 2009). On the basis of molecular, physiological and genetic studies, Cao *et al.* (2005) have detected and reported the role of gene DET2, which when over expressed resulted in the enhancement of resistance to oxidative stress in *det2* plant. The obvious result was correlated with a constitutive increase in SOD activity and increased transcript levels of the defence gene CAT. In addition to this, BRs also enhanced the expression of specific genes coding different enzymes (Khripach *et al.*, 1999), could be another reason for the elevation in the activity of POX, CAT and SOD (Tables 27-28). Similarly, BRs also enhanced the level of antioxidative enzymes and also that of proline in NaCl stressed rice (Nunez *et al.*, 2003; Ozdemir *et al.*, 2004) and mustard (Ali *et al.*, 2007), cadmium stressed mustard (Hayat *et al.*, 2007a) and chickpea (Hasan *et al.*, 2008), aluminium stressed mungbean (Ali *et al.*, 2008a)

and salinity/temperature stressed *Vigna radiata* (Hayat *et al.*, 2010a). The application of NO, at lower concentrations has also been found to enhance the activity of antioxidant enzymes (Tables 20-21). The results are also in conformity with Kanner *et al.* (1991). Moreover, the activities of SOD, POX and CAT in the presence of SNP and salt stress were much higher than those under salt stress alone. This finding was in agreement with the studies carried out by Uchida *et al.* (2002), who found that NO induced AOS activities in rice under salt stress, Guo *et al.* (2009) in *Kosteltzkya virginica*, and Hayat *et al.* (2010b) in tomato plants (Plate II).

Proline has been considered as an inert compatible osmolyte that protects subcellular structures and macromolecules under osmotic stress (Csonka and Hanson, 1991; Hare and Cress, 1997; Kavi-Kishore *et al.*, 2005). However, proline accumulation can influence stress tolerance in multiple ways. Proline has been shown to function as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes. Several studies have attributed an antioxidant feature to proline, suggesting ROS scavenging activity and proline acting as a singlet oxygen quencher (Smirnoff and Cumbes, 1989; Matysik *et al.*, 2002). Proline reduces the damaging effects of singlet oxygen and hydroxyl radicals on Photosystem II (PSII) in isolated thylakoid membranes (PSII) (Alia and Mohanty, 1997). Compromised proline accumulation in p5cs1 insertion mutants led to accumulation of ROS and enhanced oxidative damage (Szekely *et al.*, 2008). A similar effect was observed in yeast, where low proline levels in PUT1 (proline dehydrogenase)-over-expressing lines led to enhanced ROS, whereas, higher proline content in put1 mutants correlated with increased protection from oxidative damage (Chen *et al.*, 2006). As an alternative to direct ROS scavenging feature, proline can protect and stabilize ROS scavenging enzymes and activate alternative detoxification pathways. In salt-stressed

tobacco cells, proline increased the activities of methylglyoxal detoxification enzymes, enhanced peroxidase, glutathion-S-transferase, SOD and CAT activities, and increased the glutathione redox state (Hoque *et al.*, 2008; Islam *et al.*, 2009). In the desert plant *Pancratium maritimum,* CAT and POX were found to be stabilized by proline during salt stress (Khedr *et al.*, 2003). The salt hypersensitive p5cs1 *Arabidopsis* mutant shows reduced activities of key antioxidant enzymes of the glutathione ascorbate cycle, leading to hyperaccumulation of H_20_2, enhanced lipid peroxidation and chlorophyll damage (Szekely *et al.*, 2008). Accumulation of P5CS1 and P5CR in chloroplasts during salt stress suggests that, under adverse conditions, glutamate-derived proline biosynthesis increases in plastids, where photosynthesis occurs (Szekely *et al.*, 2008; Rayapati *et al.*, 1989). In mitochondria, proline has distinct protective functions. After stress, proline pools supply a reducing potential for mitochondria through the oxidation of proline by PDH and P5CDH, provide electrons for the respiratory chain and therefore, contribute to energy supply for resumed growth (Hare and Cress, 1997; Kavi-Kishore *et al.*, 2005). Proline was shown to protect Complex II of the mitochondrial electron transport chain during salt stress and therefore, stabilized mitochondrial respiration (Hamilton and Heckathorn, 2001). The recently discovered P5C–proline cycle can deliver electrons to mitochondrial electron transport without producing glutamate and, under certain conditions, can generate more ROS in the mitochondria (Miller *et al.*, 2009). Proline catabolism is, therefore, an important regulator of cellular ROS balance and can influence numerous additional regulatory pathways. Although species-specific differences in proline accumulation exist, whether differences in proline accumulation have an adaptive value, the question is still under flux. Halophyte relatives of *Arabidopsis*, such *as Thellungiella halophila* and *Lepidium crassifolium*, have elevated proline levels under unstressed

conditions and accumulate proline to higher levels than does *Arabidopsis* when exposed to high salinity (Murakeozy *et al.*, 2003; Taji *et al.*, 2004). Similar results were also reported in the present investigation (Tables 7, 14). In *Thellungiella*, high proline accumulation results from enhanced P5CS and reduced PDH expression levels (Taji *et al.*, 2004; Kant *et al.*, 2006). High proline levels can improve the salt tolerance of the halophyte plant *Pancratium maritimum*, by stabilizing detoxifying enzymes and protein turnover machinery and stimulating the accumulation of stress protective proteins (Khedr *et al.*, 2003).

The accumulation of proline in response to salinity stress is primarily localized in the cytosol of the plants (Pahlich *et al.*, 1983). Liu and Zhu (1997) found that the salt overly- sensitive *sos1 Arabidopsis* has an increased capability to accumulate proline in comparison to wild type plants. Interestingly, the enhanced resistance to salt-stress was attributed to BR-induced effects on membrane stability and osmoregulation (Wang *et al.*, 1993). BRs induced salt tolerance in rice also by enhancing metabolic turn over of nucleic acids, soluble proteins, and nitrate reductase activity (Anuradha and Rao, 2001; Anuradha and Rao, 2003).

Under water (or salinity) stress the increase in proline content (Tables 7, 14) may be due to increased hydrolysis of protein or it could be due to decreased protein biosynthesis (Irigoyen *et al.*, 1992). In the former case, protein synthetic machinery may be diverted towards the proline accumulation. Secondly, enhanced level of proline could be due to its decreased hydrolysis. Comparable observation regarding the increased content of proline have also been reported earlier in response to salt stress in *Brassica juncea* (Hayat *et al.*, 2007; Yusuf *et al.*, 2008) and *Vigna radiata* (Hayat *et al.*, in press). Also exogenously applied SNP enhanced the proline content (Tables 49, 56) significantly under salt stress. It seems that increased level of proline

has an important role in protecting enzymes involved in the antioxidant system giving protection from the salt stress (Hayat *et al.*, 2010a).

Overall, the use of EBL was more effective than HBL, alone or applied to the salt stressed plants. This is due to the variation in the structure and stability of the analogues of BRs (Khripach *et al.*, 2003). The response of seed soaking and soil application of salt reflected marked difference in their response. It appears that plants acclimate or manage to detoxify the constant dose of salinity after a due passage of time when salt given in the soil. While the direct exposure of salt during beginning of metabolism in germinating seeds proved more lethal in the present investigation. The germinating seeds have higher lipid metabolism and more anoxic environment where NO finds its pivotal role (Yamasaki and Sakihama, 2000). NO is more soluble in lipid than water and so may accumulate preferentially in membranes where its rates of reaction with any interacting molecules may be consequently higher (Liu *et al.*, 1998).

Conclusions

1. The plants of tomato (*Lycopersicon esculentum*) differentially tolerated the stress generated by varied concentrations of NaCl, applied either through seed soaking or through soil.
2. Out of the tested concentrations of NaCl (50, 100 or 150 mM) given through seed soaking, the highest concentration of salt i.e. 150 mM was found to be most toxic.
3. Out of the different concentrations of NaCl applied through soil (i.e. 0, 2.9, 5.8 or 8.7 mg Kg^{-1}), 8.7 mg Kg^{-1} of soil generated maximum stress.
4. The toxic effects of seed soaking were more pronounced over those salt stress treatments given through soil application.
5. Out of the tested concentrations (10^{-6}, 10^{-8} or 10^{-10} M), of BR analogues (HBL/EBL), 10^{-8} M proved best.

6. Out of the two BR analogues (HBL/EBL), used in the study, EBL excelled in its effect.
7. Out of the tested concentrations of SNP (10^{-4}, 10^{-5} or 10^{-6} M), 10^{-5}M was more beneficial.
8. All the morphological and photosynthetic parameters increased significantly on being treated with either BR analogues or SNP, over their respective control plants.
9. All the morphological and photosynthetic parameters decreased significantly with the increasing concentration of NaCl, applied either through seed soaking or through the soil.
10. Stress generated by the lower concentration of NaCl either through seed soaking or through the soil was completely overcome by the application of BR analogues at 60 DAS, where EBL (10^{-8} M) generated better response than HBL (10^{-8} M).
11. Stress generated by the lower concentration of NaCl either through seed soaking or through the soil was completely overcome by the application of 10^{-5} M of SNP in most of the parameters at 60 DAS.
12. Activity of enzymes (NR and CA) and all the photosynthetic attributes, increased by the application of either of the BR analogues (HBL/EBL, 10^{-8} M) / SNP (10^{-5}M) alone or as a follow up treatment to the NaCl applied as seed soaking or through the soil.
13. Antioxidant enzymes (peroxidase, catalase and superoxide dismutase) and proline content increased in response to NaCl and/or BRs/SNP. Moreover, the follow-up treatment of the NaCl treated plants with BRs/SNP showed an additive effect.

CHAPTER-6

SUMMARY

This thesis is based on the following five chapters.

Chapter 1 includes the significance of the problem entitled, "Effect of nitric oxide and brassinosteroids on the salinity induced changes in tomato (*Lycopersicon esculentum*)".

Chapter 2 represents a comprehensive review of the available literature, related with the above problems, pertaining to growth, metabolism, and yield characteristics of the plants.

Chapter 3 explaining the details of the materials and methods employed in conducting the experiments and chemical analysis of the biological material.

Chapter 4 is comprised of the tabulated data, recorded during this study, and its brief description.

Chapter 5 deals with the possible explanations for the observations, in the light of the earlier findings.

The salient features of the observations, recorded in each of the eight experiments, are summarized below:

Experiment 1

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in double distilled water (DDW), 50, 100 or 150 mM of NaCl for 8 hours. The treated seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. Twenty days after sowing (DAS), these treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS to assess growth, relative water content, photosynthetic attributes, SPAD chlorophyll, activity

of nitrate reductase and carbonic anhydrase, proline content and antioxidative enzymes. Plants showed significantly different response to the different salt concentration. 150 mM NaCl concentration was found to be the most toxic. All the above parameters except antioxidative enzymes and proline content, showed significant decrease in response to sodium chloride treatment. However, NaCl treatment resulted in a significant increase in the antioxidative enzymes and proline content and their values increased with the increasing concentration of salt.

Experiment 2

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var K-21 were sown in earthen pots containing 0, 2.9, 5.8 or 8.7 mg of NaCl/Kg of soil. These earthen pots were filled with study loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. The tomato plant showed significantly different response to different concentrations of salt. The highest level of salt (8.7 mg Kg^{-1}) was the most toxic. All the parameters except antioxidative enzymes and proline content showed a linear decrease as the level of the salt in the sol increased (2.9, 5.8 or 8.9 mg Kg^{-1} soil). The highest level of sodium chloride (8.7 mg Kg^{-1} soil) showed maximum antioxidative enzyme and that of proline content.

Experiment 3

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW, 10^{-4}, 10^{-5} or 10^{-6} M sodium nitroprusside (SNP) for 8 hours. The treated seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. After 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The plant samples

were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. Tomato plants showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 45 to 60 DAS. Treatment of SNP shows a different response and up to 10^{-5}M of SNP, most parameters increased. 10^{-6}M of SNP proved to be inhibitory.

Experiment 4

The surface sterilized seeds of tomato (*Lycopersicon eculentum*) var. K-21 were soaked in DDW for 8 hours. These seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. After 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The foliage of forty four days old seedlings were sprayed with DDW, 10^{-6}, 10^{-8} or 10^{-10} M of 28 homobrassinolide (HBL) or 24-epibrasinolide (EBL). The samples were collected at 45 and 60 DAS. The parameters studied were the same as in experiment 1. Tomato plants showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 45 to 60 DAS. The best response was obtained by 10^{-8}M of HBL/EBL. Out of the two brassinosteroid analogues (HBL/EBL), EBL was more effective than HBL.

Experiment 5

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW, 50, 100 or 150 mM NaCl for 8 hours. The treated seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The foliage of forty four day old plants was sprayed with DDW/aqueous solution of 10^{-8} M of HBL or EBL. The samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment

1. Tomato plants showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 45 to 60 DAS. The follow up treatment of either of the brassinosteroid analogues (HBL/EBL) significantly neutralized the ill effect of salt. The level of proline and the activity of antioxidative enzymes increased in response to salt and hormone treatment. Out of the two brassinosteroid analogues (HBL/EBL), EBL was more effective than HBL.

Experiment 6

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were sown in earthen pots containing 0, 2.9, 5.8 or 8.7 mg NaCl kg^{-1} soil. These earthen pots were filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS the treated seedling were subsequently transplanted to the maintained pots. The foliage of forty four day old plants was sprayed with DDW/aqueous solution of 10^{-8}M of 28 homobrassinolide (HBL) and 24-epibrasinolide (EBL). The plant samples were collected at 45 and 60 DAS to study the parameters same as in experiment 1. All the parameters increased with the progress of the plant age. The foliar spray of HBL or EBL improved the values of all the parameters and neutralized the damaging effect of the salt. EBL was better promoter than HBL.

Experiment 7

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW, 50, 100 or 150 mM NaCl for 8 hours and then transferred to the solution of DDW or 10^{-5} M of SNP for 8 hours again. The seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The samples were collected at 45 and 60 DAS.

The parameters studied were the same as in Experiment 1. Plants showed significantly different response to the treatment. All the parameters increased as the growth progressed from 45 to 60 DAS. The ill effect generated by the lowest concentration of salt was completely neutralized by SNP.

Experiment 8

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW or SNP (10^{-5}M) for 8 hours. The treated seeds were sown in earthen pots containing 0, 2.9, 5.8 or 8.7 mg NaCl Kg^{-1} soil. These earthen pots were filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS the treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS to study the characteristics studied in experiment 1. All the parameters increased with the progress of the plant age. The ill effect generated by the lowest concentration of salt was completely neutralized by SNP, whereas medium concentration of salt was partially neutralized.

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APPENDIX

1 Preparation of reagents for NRA

1.1 0.1M Phosphate buffer (pH 7.4)

27.2 g of KH_2PO_4 and 45.63 g of $K_2HPO_4.7H_2O$ were dissolved separately in 1000 ml of DDW. The above solutions of KH_2PO_4 and $K_2HPO_4.7H_2O$ were mixed in the ratio of 16:24.

1.2 0.2M KNO_3

20.2 g of KNO_3 was dissolved in sufficient DDW and final volume was made upto 1000 ml, using DDW.

1.3 5% Isopropanol

5 ml of Isopropanol was pipetted into sufficient DDW and final volume was made upto 100 ml, using DDW.

1.4 1% sulphanilamide

1 g of sulphanilamide was dissolved in 100 ml of 3N HCl. 3N HCl was prepared by dissolving 25.26 ml of HCl in sufficient DDW and final volume was maintained to 100 ml, by using DDW.

1.5 0.02% N-1-Nephthyl-ethylenediamine dihydrochloride (NED-HCl)

20 mg of NED-HCl was dissolved in sufficient DDW and final volume was made upto 100 ml, by using DDW.

2 Preparation of reagents for the estimation of carbonic anhydrase activity

2.1 Cystein hydrochloride solution 0.2M

42 g cystein hydrochloride was dissolved in sufficient DDW and final volume was made upto 1000 ml, by using DDW.

2.2 Sodium Phosphate buffer

27.2 g NaH_2PO_4 and 53.65g Na_2HPO_4 was dissolved each separately in sufficient DDW and final volume was made 1000 ml. 51 ml of NaH_2PO_4 and 49 ml of Na_2HPO_4 were then mixed to get the required solution.

2.3 Alkaline sodium bicarbonate solution

16.2 g sodium bicarbonate ($NaHCO_3$) was dissolved in aqueous 0.2M NaOH solution [0.2g NaOH $(1000\ ml)^{-1}$] and final volume was made upto 1000 ml, by using DDW.

2.4 0.002% bromothymol blue

0.002 g of bromothymol blue was dissolved in sufficient DDW and final volume was made upto 1000 ml by using DDW.

2.5 0.5 N HCl

4.3 ml of pure HCl was pipetted in sufficient DDW and final volume was made upto 1000 ml, by using DDW.

2.6 Methyl red indicator

5 mg of methyl red was dissolved in sufficient ethanol and final volume was made 100 ml, using ethanol.

3 Reagents for catalase estimation

3.1 Phospahte buffer (0.1M) for pH 6.2

3.54 g of Na_2HPO_4 was dissolved in 100 ml of DDW and 3.72 g of NaH_2PO_4 was added to 100 ml of DDW. To this 12.3 ml of Na_2HPO_4 was added to 27.7 ml of NaH_2PO_4.

3.2 H_2O_2 (0.1 M)

0.34 ml of H_2O_2 was added to 100 ml of distilled water.

3.3 Sulphuric acid H_2SO_4 (2%)

2 ml of H_2SO_4 was added to 92 ml of DDW.

3.4 0.1N potassium permanganate

This was made by dissolving 0.162 g of $KMnO_4$ in 500 ml of distilled water.

4 Reagent for peroxidase estimation

4.1 Pyrogallol phosphate buffer

It was prepared by mixing 25 ml of pyrogallol in 75 ml phosphate buffer (pH 6).

5 Reagents for superoxide dismutase

5.1 Phosphate buffer (50 mM) for pH 7.2

It was prepared by mixing 1.72 g Na_2HPO_4 and 1.56 g of NaH_2PO_4 in 100 ml of DDW separately and mixing 91.5 ml of Na_2HPO_4 with 2.5 ml of NaH_2PO_4.

5.2 Methionine (13 mM)

It was prepared by dissolving 0.193 g of methionine in 100 ml of DDW.

5.3 Nitrobluetetrazelium (NBT) (75 µM)

6.13 mg of NBT was dissolved in 100 ml of DDW.

5.4 Riboflavin (2mM)

0.732 mg of riboflavin was dissolved in 100 ml of DDW.

5.5 EDTA (0.1M)

2.92 g EDTA was dissolved in 100 ml of DDW.

6 Preparation of reagents for proline estimation

6.1 Sulphasalicylic acid (3%)

3g of sulphasalicylic acid was dissolved in sufficient DDW and final volume was maintained to100 ml, by using DDW.

6.2 Acid ninhydrin solution

1.25 g of ninhydrin was dissolved in a mixture of warm, 30 ml of glacial acetic acid and 6M phosphoric acid (pH 1.0) with agitation till it got dissolved. It was stored at 4°C and used within 24 h.

The 6M phosphoric acid was prepared by mixing 11.2 ml of phosphoric acid with 2.2 ml of DDW.